CHARLES UNIVERSITY IN PRAGUE
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Department of Pharmacology and Toxicology

Interactions between receptors in the rat mesenteric vessels

(Diploma thesis)

In cooperation with
UNIVERSIDADE DO PORTO
FACULDADE DE FARMÁCIA

Laboratório de Farmacologia

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Doc. František Trejtnar

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Interakce mezi receptory v mezenterických cévách potkana

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Ve spolupráci s
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1. INTRODUCTION

The α_2-adrenoceptors are the main $G_{i/o}$-protein coupled autoreceptors involved on the inhibition of noradrenaline release but the noradrenergic nerve terminals also possess heteroreceptors by which other transmitters and mediators influence noradrenaline release. The α_2-adrenoceptors play a key regulatory role on synaptic transmission at the postganglionic sympathetic nerve terminals not only due to its direct effects on the modulation of sympathetic transmitters release, but also by modulating effects mediated by other heteroreceptors.

The occurrence of interactions between α_2-autoreceptors and inhibitory heteroreceptors has been described in noradrenergic neurones both in the central nervous system and in peripheral tissues and, in general, activation of α_2-adrenoceptors decreases the effect mediated by other $G_{i/o}$-protein coupled receptors. The site of interaction is, in most cases, the G-protein to which both types of receptors are coupled. Interaction between α_2-autoreceptors and prejunctional facilitatory receptors has also been extensively reported in different sympathetically innervated tissues where ongoing α_2-autoinhibitionhas been shown to enhance the facilitation of noradrenaline release mediated by adenosine A_{2A},angiotensin AT_1 and bradykinin B_2 receptors and to attenuate the facilitation mediated by β_2-adrenoceptors.

The interaction between α_2-adrenoceptors and facilitatory receptors seems to take place at some step of the transduction pathways. Angiotensin AT_1 and bradykinin B_2 receptors, that requirean ongoing inhibition mediated by α_2-adrenoceptors to facilitate noradrenaline release, are coupled to the $G_{q/11}$-phospholipase C – protein kinase C pathway. It has been proposed that facilitation of noradrenaline release mediated by these receptors is caused by a PKC mediated disruption of the $G_{q/11}$-pathway to which α_2-adrenoceptors are coupled, by phosphorilation of the $G_{i/o}$-proteins, of the α_2-adrenoceptor itself or of the N-type Ca^{2+}-channel (Talaia et al., 2005).

Crosstalk between α_2-adrenoceptors and presynaptic angiotensin AT_1 receptors has been described in several tissues but the influence of presynaptic P2 receptors in such interactions, particularly in tissues where ATP is co-released with noradrenaline was investigated. Recently, one group described the occurrence of crosstalk between facilitatory A_{2A} adenosine-receptors with P2-inhibitory receptors in sympathetic innervated tissues (Fresco et al., 2002;Queiroz et al., 2003).

The aim of this work was to:

1) Investigate the influence of the A_1-inhibitory receptors on the modulation of sympathetic transmission mediated by angiotensin and adenosine, acting on A_1 receptors in vessels under conditions of different levels of ongoing inhibition mediated by adenosine and ATP

2) Elucidate the mechanisms involved in such interactions. We expected to clarify whether facilitation of noradrenaline release by angiotensin AT_1 receptors depends on, or can be correlated with, the degree of ongoing A_1- inhibition. We expected also to clarify the molecular mechanism of such interaction, namely the role of protein kinase C and protein kinase A.

2. ABBREVIATIONS

ANG II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH.2$CH_3COOH.H_2O$
CGS 21680	2-*p*-(2-Carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine hydrochloride
DPCPX	8-cyclopentyl-1,3-dipropylxanthine
SCH 58261	5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo [1,5-c]pyrimidine

3. THEORETICAL PART

3.1. ADENOSINE RECEPTORS

3.1.1. GENERAL INFORMATION

Within the superfamily of purine receptors, one has to discriminate between receptors for purine nucleotides, P2 receptors, and those for nucleosides, P1 or adenosine receptors. The adenosine receptors can be further subdivided by pharmacological means into three groups designated A_1, $-_2$, and $-_3$, and within the A_2 subgroup, one can differentiate between A_{2A} and A_{2B} receptors. All types of adenosine receptors are widely distributed in the central and peripheral nervous systems (Boehm and Kubista, 1998).

3.1.2. STRUCTURE

All adenosine receptors couple to G proteins. In common with other G protein-coupled receptors, they have seven putative transmembrane (TM) domains of hydrophobic amino acids, each believed to constitute an ahelix of approximately 21 to 28 amino acids. The N-terminal of the protein lies on the extracellular side and the C-terminal on the cytoplasmic side of the membrane. A pocket for the ligand binding site is formed by the three-dimensional arrangement of the a-helical TM domains, and the agonist is believed to bind within the upper half of this pore. The transmembrane domains are connected by three extracellular and three cytoplasmic hydrophilic loops of unequal size; typically the extracellular loop between TM4 and TM5 and the cytoplasmic loop between TM5 and TM6 is extended. These features are illustrated in a schematic of the A_1 receptor in figure 1. N-linked glycosylation often occurs on the second extracellular loop. The intracellular segment of the receptor interacts with the appropriate G protein with subsequent activation of the intracellular signal transduction mechanism. The third intracellular loop of the adenosine A_{2A} receptor seems to be the main determinant of its G protein selectivity. Phosphorylation by protein kinases of amino acid residues on the cytoplasmic domains seems to be involved in desensitization of A_{2A} and A_3 receptors. The transmembrane regions are generally highly conserved, with particularly long

stretches of amino acid homology being found in TM2, TM3, and TM5. A number of amino acid residues contribute, in different ways, to ligand specificity within the binding pocket. Sitedirected mutagenesis of the bovine A_1 adenosine receptor suggests that conserved histidine residues in TM6 and TM7 are important in ligand binding (Ralevic and Burnstock, 1998).

All four adenosine receptors have been cloned from rat, mouse, and human. In addition, A_1 receptors are cloned from dog, cow, rabbit, guinea pig, and chick; the A_{2A} receptor from dog and guinea pig; the A_{2B} receptor from chick; and the A_3 receptor from dog, sheep, rabbit, and chick. There is a close similarity between receptors of the same subtype. The largest variability is seen for the A_3 receptor for which there is almost a 30% difference at the amino acid level between human and rat. This difference is in fact larger than that between human and chick A_1 receptors. It has long been known that A_1 and A_3 receptors couple to $G_{i/o}$ and that A_{2A} and A_{2B} receptors couple to G_s. Experiments with chimeric A_1/A_{2A} receptors indicate that structural elements in both the third intracellular loop and the carboxyl terminus influence coupling of A_1 receptors to G_i, whereas elements in the third intracellular loop but not the carboxyl terminus contribute to A_{2A} receptor coupling to G_s (Fredholm et al., 2001).

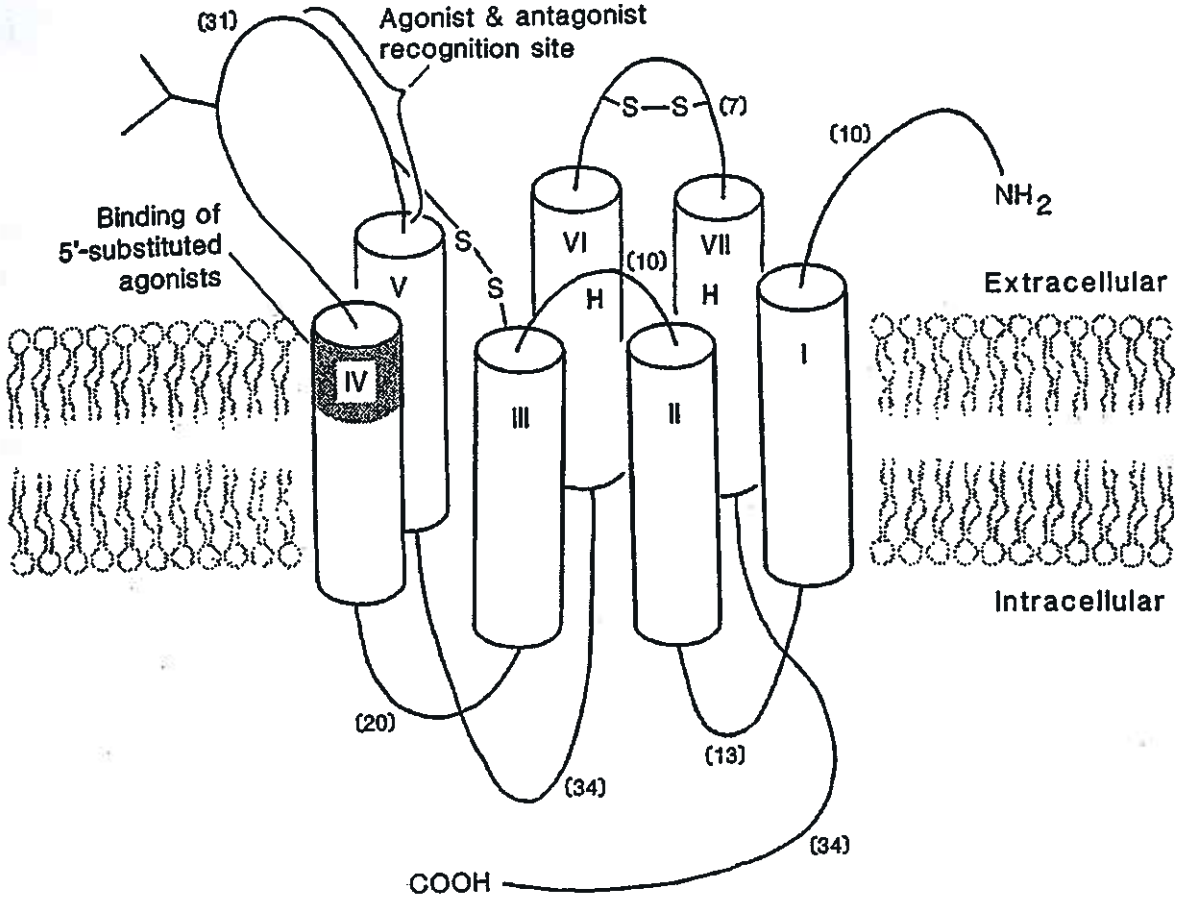

Fig. 1: Schematic picture of the A_1 adenosine receptor. Like other G protein-coupled receptors, the A_1 receptor has seven putative transmembrane domains (I-VII) of hydrophobic amino acids, each believed to constitute an α-helix, which are connected by three extracellular and three intracellular hydrophilic loops. The number of amino acids comprising the extra- and intracellular loops and the extracellular N-terminal and intracellular C-terminal regions of the bovine A_1 receptor are indicated in parentheses. The transmembrane regions comprise 23 to 25 amino acids in the bovine A_1 receptor. The arrangement of the transmembrane regions forms a pocket for the ligand binding site. The locations of histidine residues (H) in transmembrane regions VI (position 254) and VII (position 278) in the bovine A_1 receptor, which are believed to be important in ligand binding, are indicated. Extracellular and transmembrane regions of the protein believed to be important in agonist and antagonist binding are indicated. S-S denotes the presence of hypothetical disulfide bridges. Glycosylation occurs on the second extracellular loop. Adapted from Ralevic and Burnstock 1998.

3.1.3. DISTRIBUTION

It is important to study the distribution of receptors, because this will tell us where agonists and antagonists given to the intact organism can act. Furthermore, in general, the higher the number of receptors the more potent and/or efficacious will be the agonist. Thus, the rather low levels of endogenous adenosine present under basal physiological conditions have the potential of activating receptors where they are abundant. There is much information on the distribution of the A_1 and A_{2A} receptors because good pharmacological tools including radioligands are available. There are also several studies that have used antibodies to localize adenosine A_1 receptors in brain, and A_{2A} receptors in striatum, carotid body, and T cells. In the case of the A_{2B} and A_3 receptors, the data are less impressive. Here one tends to rely on data on the expression of the corresponding mRNA. Some of this information is summarized in the table below. The results presented there clearly show that there is much left to examine regarding the distribution of especially A_{2B} and A_3 receptors. Furthermore, it is likely that a better understanding of the transcriptional regulation will be of considerable help in understanding the spatio-temporal aspects of adenosine receptor distribution. Receptor protein and the corresponding message are often colocalized but there are important differences. For example, in several regions of the central nervous system, receptor binding and expression of transcript do not exactly match, and the two are differently regulated by e.g., long term antagonist treatment and during development. Much of the differential distribution can probably be explained by the fact that a substantial number of adenosine A_1 receptors are present at nerve terminals. A similar explanation probably underlies the observations that A_{2A} receptors are present in globus pallidus, despite the fact that A_{2A} receptor mRNA cannot be detected there. These receptors are probably located at the terminals of the striatopallidal GABAergic neurons. Besides regulation at the level of gene transcription, targeting of the receptor protein to different locations within the cell is crucial. This important aspect of receptor distribution is just starting to be explored in the case of adenosine receptors. The distribution is highly dependent on the third intracellular loop and/or the carboxyl-terminal segment of the receptor as judged by the distribution of receptor chimeras. Thus, G protein coupled receptors appear to use several different targeting mechanisms (Fredholm et al., 2001).

Table 1: Summary of distribution of adenosine receptors (Adapted from Fredholm et al., 2001)

A_1	A_{2A}	A_{2B}	A_3
High expression: Brain (cortex, cerebellum, hippocampus). Dorsal horn of spinal cord. Eye, adrenal gland, atria	**High expression:** Spleen, thymus, leukocytes (both lymphocytes and granulocytes), blood platelets. Striatopallidal GABAergic neurons (in caudate-putamen, nucleus accumbens, tuberculum olfactorium), olfactory bulb	**High expression:** Cecum, colon, bladder	**High expression:** Testis (rat), mast cells (rat)
Intermediate levels: Other brain regions. Skeletal muscle, liver, kidney, adipose tissue, salivary glands, esophagus, colon, antrum, testis	**Intermediate levels:** Heart, lung, blood vessels	**Intermediate levels:** Lung, blood vessels, eye, median eminence, mast cells	**Intermediate levels:** Cerebellum (human?), hippocampus (human?), lung, spleen (sheep), pineal
Low levels: Lung, pankreas	**Low levels:** Other brain regions	**Low levels:** Adipose tissue, adrenal gland, brain, kidney, liver, ovary, pituitary gland	**Low levels:** Thyroid, most of brain, adrenal gland, spleen (human), liver, kidney, heart, intestine, testis (human)

3.1.4. SIGNALING MECHANISM

3.1.4.1. A_1 RECEPTOR

The A_1 receptor mediates a broad range of signaling responses, which may be caused by its coupling to different G proteins within the $G_{i/o}$ family. The G proteins G_i and G_o are substrates for pertussis toxin that ADP-ribosylates the α-subunit of $G_{i/o/t}$ family members, uncoupling them from receptors. Accordingly, effects mediated by A_1 receptors are generally blocked by pertussis toxin. However, presynaptic A_1 receptors seem to be at least partly resistant to pertussis toxin; the reason for this could be the very tight coupling of the presynaptic A_1 receptors to potentially pertussis toxin-sensitive G proteins, rather than coupling to pertussis toxin-insensitive G proteins.

The most widely recognized signaling pathway of A_1 receptors is inhibition of adenylate cyclase causing a decrease in the second-messenger cAMP. This in turn modulates the activity of cAMP-dependent protein kinase, which phosphorylates diverse protein targets. A_1 oupling to adenylate cyclase has been described in a number of tissues including brain, adipose tissue, and testes. In addition to direct modulation of signaling pathways downstream to cAMP, inhibition of adenylate cyclase via A_1receptors blocks the effects of other agents which act by stimulating adenylate cyclase activity in cells.

Another signaling mechanism of A_1 receptors is activation of phospholipase C (PLC) leading to membrane phosphoinositide metabolism and increased production of inositol 1,4,5-triphosphate (IP3) [and diacylglycerol (DAG)] and Ca^{2+} mobilization. This has been described in chinese hamster ovary (CHO)-K1 cells expressing the cloned human A_1 receptor as well as at endogenous A_1 receptors in a number of tissues including DDT1 MF-2 smooth muscle cells, heart, myometrium, rabbit cortical collecting tubule cells, renal cells, tracheal epithelial cells, cultured mesangial cells, and primary astrocytes. IP3 stimulates the release of Ca^{2+} from intracellular stores via interactions with specific receptors located on the sarcoplasmic reticulum. Elevation of cytosolic Ca^{2+} by IP3 can stimulate a variety of signaling pathways, including a family of phosphatidyl serine-dependent serine/threonine-directed kinases collectively called protein kinase C (PKC) (of which there are at least 11 different isoforms), phospholipase A2 (PLA2), Ca^{2+}-dependent K1 channels, and nitric oxide synthase (NOS). Depletion of Ca^{2+} from IP3-sensitive pools may promote Ca^{2+} influx from extracellular sources.

Activation of phospholipase D (PLD) via A_1 adenosine receptors in DDT1 MF-2 smooth muscle cells has been described, although as in the majority of cell systems this may be downstream of phosphoinositide hydrolysis and may require the intermediate activation of PKC or Ca^{2+} (Ralevic and Burnstock, 1998).

Stimulation of A_1 receptors can activate several types of K1 channel, described principally in cardiac muscle and neurons. In supraventricular tissues (sino-atrial and atrioventricular node, and atrium), the A_1 receptor couples directly via pertussis toxin-sensitive G proteins to K1 channels (the same K1 channels are activated by both adenosine and acetylcholine), and activation causes bradycardia. A_1 adenosine receptors also couple to ATP-sensitive K1 channels (KATP channel); the activity is additionally regulated by metabolic demand (they close when intracellular ATP levels are high). Coupling seems to occur through the G protein in a membrane-delimited manner, although coupling via cytosolic factors is possible given the strong evidence that A_1 receptors, KATP channels, and PKC all have a role in ischemic preconditioning. A_1 receptor coupling to KATP channels has been described in rat and guinea-pig ventricular myocytes, porcine coronary arteries, rabbit heart, and rat cerebral cells. Activation of KATP channels mediates a reduction in action potential duration, vasodilatation and an increase in blood flow, which is consistent with their having a pivotal role in the coupling of vascular tone to metabolic demand determined both by intracellular purines (ATP/ADP levels) and by the extracellular actions of adenosine (released, for instance, during hypoxia or ischemia). Neurons express multiple K1 channels that A_1 receptors may couple to regulate membrane potential and determine action potential frequency and duration. A_1 receptors reduce neuronal excitability and decrease firing rate by a hyperpolarizing effect mediated mainly by an increase in K1 conductance. A_1 receptors also couple to inhibition of Ca^{2+} currents, which may account for inhibition of neurotransmitter release, although other or multiple mechanisms may be involved in this process. Inhibition of Ca^{2+} currents by A_1 receptors has been described in dorsal root ganglion neurons, rat hippocampal pyramidal neurons, rat sympathetic neurons (N-type Ca^{2+} channels, plus an unidentified Ca^{2+} channel), rat brainstem (predominantly N-type, but also P-type Ca^{2+} channels), hippocampal CA1 neurons (N-type, plus some unidentified Ca^{2+} channels), hippocampal CA3 neurons (N-type Ca^{2+} channel), and mouse motoneurons (N-type Ca^{2+} channel). In atrial myocytes adenosine has an inhibitory effect on basal L-type Ca^{2+} current, although this is small and may be secondary to a reduction in basal cAMP (Ralevic and Burnstock, 1998).

3.1.4.2. A_{2A} RECEPTOR

The most commonly recognized signal transduction mechanism for A_{2A} receptors is activation of adenylate cyclase. This implies coupling with the G protein Gs, although other G proteins may also be involved. Vibrio cholerae (cholera toxin) ADP-ribosylates the α-subunit of G_s family members, inhibiting the intrinsic GTPase activity of Gαs and thus has been useful in characterizing members of this family. Coupling of the A_{2A} receptor to its G protein is tight. Hence, there is only slow dissociation of agonist from the receptor and stabilization of the receptor-G protein complex.

cAMP-independent signaling has been suggested for A_{2A} receptors on striatal GABA nerve terminals and striatal cholinergic nerve terminals. In striatal nerve terminals, A_{2A} receptors are suggested to mediate dual signaling via P- and N-type Ca^{2+} channels linked to G_s/adenylate cyclase/PKA and cholera toxin-insensitive G protein/PKC, respectively. It has been suggested that A_{2A} receptor-mediated inhibition of superoxide anion generation in neutrophils may be mediated via cAMP-independent activation of a serine/threonine protein phosphatase. A_{2A} receptor-mediated facilitation of synaptic transmission and transmitter release seems to occur through potentiation of presynaptic P-type Ca^{2+} channels, and probably involves adenylate cyclase and activation of a cAMP-dependent protein kinase.

KATP channels are suggested to be involved in coronary vasodilatation mediated by A_2 receptors in the dog. Activation of KATP channels by A_2 receptors in arterial myocytes is suggested to involve a cAMP-dependent protein kinase (Ralevic and Burnstock, 1998).

3.1.4.3. A_{2B} RECEPTOR

A_{2B} receptor coupling to different signaling pathways has been reported, including activation of adenylate cyclase, $G_{q/11}$-mediated coupling to PLC and IP3-dependent increase in $[Ca^{2+}]$ (in human mast cells), and coupling to PLC when expressed in Xenopus oocytes (Ralevic and Burnstock, 1998).

3.1.5. AGONISTS

3.1.5.1. A_1 RECEPTOR

Certain N6-substituted adenosine derivatives, such as N6-cyclopentyladenosine (CPA), N6-cyclohexyladenosine (CHA), and R-PIA, are selective agonists at A_1 receptors. Substitutions at both the N6- and C2-positions have produced 2-chloro-CPA (CCPA) which is A_1 selective, 1500-fold versus A_2 receptors in binding studies in rat brain. N-[1S, trans,2-hydroxycyclopentyl] adenosine (GR79236) has been reported to be an A_1 selective agonist, which is approximately equipotent with CPA in a variety of isolated tissues and cell types (Ralevic and Burnstock, 1998).

3.1.5.2. A_{2A} RECEPTOR

A_{2A} receptors do not generally bind N6-substituted adenosine derivatives and show a preference for derivatives with modifications of the 2nd position of the adenine ring; bulky substituents in this position can selectively enhance A_{2A} receptor affinity. Several synthetic A_{2A}-selective agonists are modeled according to this structural modification. It should be noted that the agonist studies detailed below have been carried out in species other than humans, and that the human A_{2A} receptor has a comparatively lower affinity of binding for CGS 21680 and other adenosine receptor agonists.

The C2-substituted NECA derivative, CGS 21680, is 140-fold selective for the A_{2A} versus the A_1 receptor. CGS 21680 has only very low affinity at the A_{2B} receptor, and thus has been used extensively to discriminate between A_{2A} and A_{2B} subtypes. [^{3}H]CGS 21680 has been reported to bind in rat cortex and hippocampus to adenosine binding sites different to the classic striatal A_{2A} receptors, which does not seem to be caused by high and low affinity states of the same A_{2A} receptor, or to binding at A_3 or A_4 receptors. Amine derivatives of CGS 21680, namely APEC, DITC-APEC and 2-[4-(2-([4-aminophenyl]methylcarbonyl)-ethyl)-phenyl]ethylamino-59-N-ethylcarboxamidoadenosine (PAPA-APEC), are A_{2A}-selective agonists. DITC-APEC is binding covalently and though causing irreversible activation of the A_{2A} receptor.

The C2-substituted adenosine derivative CV 1808 displays poor selectivity (approximately 5-fold) for the A_{2A} versus the A_1 receptor, but is a valuable precursor for the synthesis of more selective A_{2A} receptor agonists. N6-(2(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl)-adenosine (DPMA) is a selective A_{2A} receptor agonist.

A series of 2-aralkynyl and 2-heteroalkynyl derivatives of NECA have been studied for their selectivity at the A_{2A} receptor. Of these, the 4-formylphenylethynyl derivative shows affinity in the low nanomolar range and approximately 160-fold selectivity. 2-Hexyl-59-N-ethylcarboxamidoadenosine (2HENECA) has been suggested to be selective at A_{2A} receptors with 60- and 160-fold selectivity in binding studies for A_{2A} versus A_1 receptors in rat and bovine brain, respectively. Although NECA itself is approximately equipotent at A_1 and A_{2A} receptors, it can be useful in A_{2A} receptor characterization provided that A_1-selective ligands are shown not to have equivalent effects.

The 2-hydrazinoadenosine, WRC-0470 (2-cyclohexylmethylidenehydrazinoadenosine) has been shown to be a potent and selective A_{2A} agonist, with low nanomolar affinity at recombinant A_{2A} receptors transfected in mammalian cells and in functional assays in a variety of tissues (Ralevic and Burnstock, 1998).

3.1.6. ANTAGONISTS

3.1.6.1. A_1 RECEPTOR

Most of the selective A_1 receptor antagonists described to date are xanthine-based derivatives. The introduction of hydrophobic (particularly phenyl or cycloalkyl) substituents into position 8 of the xanthine ring has yielded potent and A_1-selective antagonists, including 1,3-dipropyl-8-phenyl(2-amino-4-chloro)xanthine (PACPX), DPCPX, and xanthine amine congener (XAC). Of these, DPCPX has the greatest affinity (Ki 1.5 nM) for A_1 receptors and the greatest A_1-subtype selectivity (A_2/A_1 affinity ratio 740), as shown in rat brain membranes. The human A_1 receptor has an approximately lower affinity for DPCPX.

A number of other 8-substituted xanthines, including (6)-8-(3-oxocyclopentyl)-1,3-dipropylxanthine (KFM 19) and KW-3902 (8-noradamant-3-yl-1,3-dipropylxanthine), have been shown to be selective antagonists at A_1 receptors. The alkylxanthine 1,3-dipropyl-8-[2-(5,6-epoxy) norbornyl]xanthine (ENX) is a potent (KB 3.6 nM) and selective antagonist at A_1

receptors in the guinea- pig heart and brain and in DDT1 MF-2 cells, with 400-fold greater affinity of binding versus A_{2A} receptors in guineapig brain.

Several classes of non-xanthine antagonists have been described, some showing reasonable affinity and selectivity for the A_1 receptor. Some of the more active of these are the tricyclic non-xanthine antagonists, including the triazoloquinazolines, the triazoloquinoxalines, and the imidazoquinolines.

The adenine derivative 1,3-dipropyl-8-[2,(5,6-epoxy)norbornyl] xanthine (N 0861) is reasonably selective (10- to 47-fold versus A_{2A} receptors) and potent at A_1 receptors in a number of tissues. This compound has been superceded by the S-enantiomer 12 (CVT-124) with nanomolar selectivity and 1800- and 2400-fold selectivity at rat and cloned human A_1 receptors, respectively, and by 8-(N-methylisopropyl)amino-N6-(59-endohydroxy-endonorbornyl-)9-methyl adenine (WRC 0571) with 62-fold selectivity versus the A_{2A} receptor and 4670-selectivity versus the A_3 receptor.

(+)-(R)-[(E)-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)acryloyl]-2-piperidine ethanol, FK 453, has been reported to be a potent and selective A_1 receptor antagonist with IC50 values of approximately 17 nM at rat cortical A_1 receptors and 11 mM at striatal A_2 receptors. Chiral pyrolo[2,3-d]pyrimidine and pyrimido[4,5-b]indole derivatives have been shown to be potent and highly stereoselective A_1 adenosine receptor antagonists (Ralevic and Burnstock, 1998).

3.1.6.2. A_{2A} RECEPTOR

Several antagonists selective for the A_{2A} receptor have been synthesized. 8-(3-chlorostyryl)caffeine (CSC) is a potent (Ki 54 nM) and selective A_{2A} antagonist in radioligand binding assays in rat brain (520-fold selective versus A_1 receptors), in reversing agonist effects on adenylate cyclase in PC12 cells (22-fold selective), and in blocking locomotor depression elicited by the A_{2A}-selective agonist APEC in vivo. 1,3-dialkyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthine (KF-17837) has been described as a potent and selective A_{2A} antagonist with 62-fold selectivity for A_{2A} over A_1 receptors in binding studies in rat brain, and 30-fold selectivity for the A_{2A} over the A_{2B} receptor in inhibition of cAMP accumulation (A_{2A} IC_{50} = 53 nM; A_{2B} IC_{50} = 1500 nM). DMPX (3,7-dimethyl-1-

propargylxanthine) derivatives have been shown to be potent and selective A_{2A} antagonists; 8-(m-bromostyryl)-DMPX has a Ki value of 8.2 nM and is 146-fold selective versus A_1 receptors.

ZM 241385, (4-(2-[7-amino-2-(2-furyl)[1,2,4]-triazolo [2,3-a] [1,3,5]triazin-5-yl amino]ethyl)phenol) is a potent and selective non-xanthine A_{2A} adenosine receptor antagonist. It has high affinity for the A_{2A} receptor (pA2 value approximately 9), is 1000- and 91-fold selective versus A_1 and A_{2B} receptors, respectively, and has virtually no effects at A_3 receptors.

[^{3}H]SCH 58261 ([3H-5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c] pyrimidine) is a novel potent and selective A_{2A} antagonist radioligand which binds with low nanomolar affinity to A_{2A} receptors in human platelet and rat striatal membranes, and at A_{2A} receptors transfected into CHO cells. The analog SCH 63390 (5-amino-7-(3-phenylpropyl)-2-(2-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine) has similar potency at A_{2A} receptors, but greater selectivity (210-fold) (Ralevic and Burnstock, 1998).

3.2. α_2 RECEPTORS

Presnaptic receptors were discovered in 1971. The first 10 years after 1971 yielded the basic evidence. Carlsson coined the term „autoreceptors", receptors „sensitive to the neuron's own transmitter substance". The α_2-autoreceptors were the first in which an inhibitory effect of the transmitter itself was demonstrated.

The study of α_2-autoreceptors also showed that they were not one single receptor type, they differed between rats and rabbits. Radioligand binding experiments then indicated that there were four pharmacologically distinct α_2-autoreceptors, α_{2A}, α_{2B}, α_{2C} and α_{2D}. Part of the pharmacological difference can be attributed to a point mutation in the fifth transmembrane domain of this receptor where cysteine in humans is replaced by serine in mice and rats.

The qeustion arose to which subtype or subtypes the presynaptic α_2-autoreceptors belonged. Comparison of antagonist potencies at the autoreceptors with their affinities for prototypical α_{2A}, α_{2B}, α_{2C} and α_{2D} binding sites indicated that presynaptic autoreceptors were at least

predominantly α_{2A} in humans and rabbits and α_{2D} in rats and mice, i.e., belonged to the genetic $\alpha_{2A/D}$ branch with its two orthologous varieties, or in other words: mammals seemed to express mainly the $\alpha_{2A/D}$ gene in noradrenergic neurons to bring these neurones under α_2-autoreceptor control (Starke, 2001).

3.2.1. SIGNAL TRANSDUCTION

Soon after the discovery of presynaptic α_2-autoreceptors, the idea emrged that they modified calcium movements in noradrenergic terminal axons. With the discovery that all α_2-autoreceptors couple to heterotrimeric G-proteins, the first signal transduction step was also identified. Much of the presynpatic α_2-adrenergic inhibition is blocked by pertussis toxin, but some inhibition may also operate through pertussis toxin-insensitive G-proteins (Starke, 2001). The studies revealed that the α_2-autoreceptors preferentially interacts with the G_i protein familyand to a lesser extent with G_s and G_q (Chabre et al., 1994). The α_2-adrenergic inhibition of noradrenaline releasefrom chick cultured sympathetic neurones operated only as long as release was due to calcium entry through N-type channels, suggesting that presynaptic N-type calcium channels were the only target of the α_2-autoreceptor-G-protein signal transduction pathway.

The conclusions so far were drawn from noradrenaline release studies. More details have been suggested by studies on soma-dendritic α_2-autoreceptors, most frequently in the rat superior cervical ganglion, because only the soma-dendritic receptor mechanism are accessibleto electrophysiological measurements. These studies have led to the following hypothesis. In rat superior cervical sympathetic ganglion cells, agonist activation of soma-dendritic α_2-autoreceptors leads primarily to activation of G_o or G_i, i.e. dissociation of G_o into $G_{o\alpha}$ and the corresponding $\beta\gamma$–complex and dissociation of G_i into $G_{i\alpha}$ and the corresponding $\beta\gamma$–complex. The $\beta\gamma$–complexes then are more important than the G_α subunits in continuing signal transduction. The $\beta\gamma$–complexes interact directly, without intercalation of a cytoplasmic second messenger, with the pore-forming α_1 subunits of N- and P/Q-type calcium channels, thus reducing the channel's open probability. The α_2-adrenoceptor-G_i pathway seems to predominate;it is pertussis toxin sensitive, rapid and voltage-dependent: depolarisation alleviates the block of the calcium channel. The α_2-adrenoceptor-G_i pathway is pertussis toxin-resistant, slow and voltage-insensitive. The specific $G_{o\alpha}$ proteins in rat superior

cervical ganglion cells seem to be $G_{o\alpha A}$ and $G_{o\alpha B}$, and the specific $G_{i\alpha}$ protein seems to be $G_{i\alpha 2}$. Whether the same α_2-adrenoceptor subtype couples to both G_o and G_i, or whether different subtypes couple to each G-protein selectively, is not known. In cultured mouse post-ganglionic sympathetic neurones, pertussis toxin blocked both the $\alpha_{2A/D}$-adrenoceptor mediated and the non-$\alpha_{2A/D}$-adrenoceptor mediated inhibition of calcium currents.

However fascinating this elaborate hypothesis is, it should be remembred that it is based on observations on somadendritic receptors. If applicable to presynaptic autoreceptors, it might explain for example why the inhibition of noradrenaline release through α_2-autoreceptors (as well as presynaptic heteroreceptors such as cannabinoid receptors) declines at high frequencies: high frequency depolarisation might remove the voltage-sensitive part og the $\beta\gamma$ blockade of N-type calcium channels. However, some observations on presynaptic α_2-adrenergic inhibition are hard to understand on the basis of any inhibition of calcium entry through voltage-sensitive channels. Other possible modes of inhibition such as direct interference with the exocytotic machinery should be kept in mind (Starke, 2001).

3.3. MECHANISM OF INTERACTION

The mutual inhibitory interaction between the two presynaptic ligand-gated cation channels, nicotinic acetylcholine receptors and ATP P2X receptors, must be assumed to be mediated by direct protein-protein interactions. First, the interaction occurs instantaneously, thus, being too fast to involve second messenger cascades or phosphorylation/dephosphorylation reactions. Second, the interaction can also be observed in cell-free membrane patches, which verifies that intracellular signaling components are not required. Third, the interaction between nicotinic receptors and P2X receptors occurs also when the receptors are heterologously expressed in Xenopus oocytes. In contrast to this presumed protein-protein interaction, the regulation of the functions of ionotropic receptors via metabotropic receptors involves second messenger cascades. The best evidence to support this conclusion comes from electrophysiological experiments in which, for instance, prostaglandin E2 was applied to sympathetic neurons in a manner, that it could not reach the membrane area where the activity of nicotinic acetylcholine receptors was determined. Nevertheless, the eicosanoid did inhibit the frequency of channel opening. Furthermore, the inhibition of nicotinic receptors via NPY receptors was shown to involve cyclic AMP and

cyclic AMP-dependent protein kinase. Finally, the modulation of P2X receptors by adenosine was abolished by pertussis toxin implicating the involvement of inhibitory G proteins. Thus, the cross-talk from metabotropic onto ionotropic receptors appears to involve typical G proteindependent signaling cascades depending on the type of metabotropic receptor being activated.

Although ionotropic receptors do not directly affect the functions of presynaptic metabotropic receptors, they may interfere with the inhibition of transmitter release via G proteins. As mentioned above, receptor-dependent presynaptic inhibition is mediated in most cases by a closure of voltage-gated Ca^{2+} channels via G proteins. Activation of presynaptic transmitter-gated cation channels, such as nicotinic acetylcholine receptors and ATP P2X receptors, triggers sympathetic transmitter release independent of voltagegated Ca^{2+} channels. Hence, when sympathetic transmitter release is elicited by activation of these receptors, presynaptic G protein-coupled receptors can no longer reduce transmitter output through a blockade of Ca^{2+} channels.

The majority of interactions occur between two different types of metabotropic receptors, and these receptors may utilize a plethora of signaling cascades. Therefore, these interactions can involve diverse signaling mechanisms that finally converge to precisely control the amount of sympathetic transmitter being released. As mentioned above, activation of presynaptic β-adrenoceptors may facilitate sympathetic transmitter release most likely via the synthesis of cyclic AMP. This action may be antagonized by the activation of receptors that inhibit adenylyl cyclases, such as α_2-adrenoceptors, even though this signaling mechanism is not involved in the inhibition of transmitter release via these receptors. In support of this competition between β- and α_2-adrenoceptors at the level of adenylyl cyclases and cyclic AMP, it has been reported that increases in cyclic AMP raised noradrenaline release only when α_2-adrenoceptors were blocked.

Competition between the cyclic AMP-dependent signaling cascade and release inhibiting receptors may also occur at the level of voltage-gated Ca^{2+} channels. In chicken sympathetic neurons, an increase in cyclic AMP was found to counteract the inhibition of Ca^{2+} currents and of transmitter release, both via α_2-adrenoceptors. This interaction did not arise at the level of adenylyl cyclases, because α_2-adrenoceptor activation failed to inhibit adenylyl cyclase activity. Hence, an activation of cyclic AMP-dependent signaling cascades, whether by receptor or direct adenylyl cyclase activation, may not only raise transmitter release by a mechanism downstream of Ca^{2+} entry, but may also attenuate the action of receptors that inhibit release. An additional competition between presynaptic receptors that

stimulate transmitter release via increases in cyclic AMP and receptors that inhibit transmitter release has been detected at the level of G proteins. Whereas the first type of receptor operates through G_s proteins, the latter type of receptor typically involves pertussis toxinsensitive G proteins. The removal of α-subunits of $G_s$type G proteins from chicken sympathetic neurons in primary cell cultures caused sensitization of the α_2-adrenoceptor-mediated inhibition of transmitter release. This effect did not involve cyclic AMP nor changes in any G protein subunit other than α_s. Hence, the loss of stimulatory G protein α-subunits led to a sensitization of the inhibitory G protein-dependent signaling pathway. This indicates that G protein α_s-subunits may mediate a tonic attenuation of the signaling cascade that is involved in the receptor-dependent presynaptic inhibition of sympathetic transmitter release.

M1 muscarinic receptors, B2 bradykinin, and AT1 angiotensin receptors facilitate sympathetic transmitter release most likely through an activation of protein kinase C. Frequently, this facilitation was lost when presynaptic α_2-autoreceptors were blocked, i.e., when ongoing autoinhibition of transmitter release via these receptors was prevented. These results can be interpreted in at least two different ways. Either the activation of receptors linked to protein kinase C prevents the inhibitory action of α_2-adrenoceptors, or α_2-adrenoceptor activation is required to support the facilitatory effect of receptors linked to protein kinase C. The first type of interaction can be explained by results concerning the G protein-dependent modulation of voltage-gated Ca^{2+} channels. Activation of protein kinase C has been shown to prevent the inhibitory interaction of G protein βγ-subunits with N-type Ca^{2+} channels and, thus, abolishes the reduction of Ca^{2+} currents in sympathetic neurons via, for instance, α_2-adrenoceptors and somatostatin receptors. Because closure of voltage-gated Ca^{2+} channels is believed to be the crucial step in presynaptic inhibition, this effect will impede the receptor-dependent inhibition and, thus, lead to an increase in transmitter release. The second type of interaction can be explained at the level of phospholipases C. These enzymes may be activated by G protein α-subunits of the G_q family and by βγ-subunits. These latter types of G protein subunits may derive from pertussis toxin-sensitive and -insensitive heterotrimers, which may be activated by a large variety of different metabotropic receptors. Hence, α_2-adrenoceptor-dependent liberation of G protein βγ-subunits may synergize with a G_q-dependent activation of phospholipase C and, thereby, support the facilitatory effects mediated by receptors linked to this latter family of G proteins.

Interactions between two different inhibitory presynaptic receptors colocalized on sympathetic axon terminals typically occur in the way that activation of one of these receptors prevents the inhibitory action of the other one. This can again be interpreted in at least two ways. Either one of these inhibitory receptors interrupts the signaling cascade of the other one, or these two receptors share one common signaling cascade that becomes maximally stimulated by activation of only one of these two receptors. As stated above, there is good evidence to suggest that the G protein-mediated inhibition of voltage-gated Ca^{2+} channels is the predominant mechanism underlying receptor-dependent presynaptic inhibition. Considerable number of different receptors employ pertussis toxin-sensitive signaling cascades to block N-type Ca^{2+} channels, and N-type Ca^{2+} channels are the predominant channel subtype involved in excitation-secretion coupling in sympathetic axon terminals. Activation of two such receptors at the same time does not cause more inhibition of Ca^{2+} currents than the activation of one receptor only, as shown, for instance, for α_2-adrenoceptors and somatostatin receptors in chicken sympathetic neurons. This appears even more remarkable as these two receptors use different signaling cascades to inhibit the Ca^{2+} channels: the inhibitory effect of α_2-adrenoceptor activation involves an atypical, phorbol ester-insensitive protein kinase C, whereas somatostatin receptor activation does not. At the level of noradrenaline release, there is again no additivity of the inhibitory actions mediated by these two receptors. Thus, the signaling cascades of different inhibitory presynaptic receptors appear to converge at the level of voltage-gated Ca^{2+} channels, which mediate the transmembrane Ca^{2+} entry required for triggered vesicle exocytosis. Therefore, activation of one of these inhibitory receptors will occlude an inhibitory action of another one (Boehm and Kubista, 2002).

4. MATERIALS AND METHODS

4.1. PREPARATION AND EXPERIMENTAL CONDITIONS

Adult male Wistar rats (250–300 g; IBMC, Porto, Portugal) were kept at a constant temperature (21 °C) and a regular light (06.30–19.30 h)/dark (19.30–06.30 h) cycle, with food and water ad libitum. Animal handling and experiments with animals were conducted according to the guidelines of the European Communities Council Directive (86/609/EEC). Animals were killed after stunning followed by exsanguination. The tissues were dissected out, cleaned of connective tissue and divided in portions of about 2-3 mg weight. Tissue preparations were incubated and superfused, at 37 °C, with gassed (95% O2 and 5% CO2) Krebs solution of the following composition (mM): NaCl 118.6, KCl 4.70, $CaCl_2$ 2.52, $MgSO_4$ 1.23, $NaHCO_3$ 25.0, glucose 10.0, ascorbic acid 0.3 and disodium EDTA 0.031.
A Ca^{2+}-free solution was prepared by equimolar replacement of $CaCl_2$ with NaCl (Talaia et al., 2005).

4.2. EXPERIMENTAL PROTOCOL

The procedures used to label tissue preparations with [^{3}H]-noradrenaline and to estimate changes on electrically evoked tritium overflow as an indicator of changes on neuronal noradrenaline release, have been previously described (e.g. Queiroz et al., 2003, 2004). Briefly, tissue preparations of the prostatic portion of rat vas deferens were pre-incubated in 2 ml of Krebs solution containing [^{3}H]-noradrenaline (0.1 mM; specific activity of 53.0 Ci $mmol^{-1}$) for 40 min. Tissue preparations were then transferred to superfusion chambers where they were held between platinum electrodes 7 mm apart by a polypropylene mesh, and superfused with [^{3}H]-noradrenaline free medium at a constant flow rate of 1 ml min^{-1}. A stimulator (Hugo Sachs Elektronic, Type 215, March-Hungstetten, Germany), operating in the constant current mode, was used for electrical field stimulation with square wave pulses (1 ms width; 50 mA current strength; voltage drop of 18 V per chamber). The stimulation periods consisted of 100 or 200 pulses at 2 Hz, 200 pulses at 5Hz, 100 pulses at 8 Hz or 20 pulses at 50 Hz. A primer stimulation period, applied at t = 30 min (t = 0 min being

the onset of superfusion) was not used for determination of tritium overflow. Subsequent stimulation periods were applied at t = 60 min (S_1), t = 90 min (S_2), t = 120 min (S_3) and t = 150 min (S_4) and t = 180 min (S_5). In some experiments the interval between the S_n was only 20 minutes. In some experiments only S_1 and S_2 were applied. Superfusate samples were collected at 5 min intervals, from t = 55 min onwards. At the end of the experiments, tritium content was determined in superfusate samples and in tissue preparations by scintillation spectrometry (Beckman LS 6500, Beckman Instruments, Fullerton, USA). Desipramine (0.4 mM; noradrenaline neuronal uptake inhibitor) and in some experiments 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 0.1 mM; adenosine A_1 receptors antagonist) were present from the beginning of superfusion and kept throughout, unless otherwise stated. Yohimbine, was in some experiments added at the beginning of superfusion, and kept throughout. (Talaia et al., 2005).

4.3. INTERPRETATION OF DRUG EFFECTS ON EVOKED TRITIUM OVERFLOW

The tritium outflow was estimated as fraction of the tissue tritium content at the onset of the respective collection period (fractional rate of outflow, min^{-1}). Drug effects on basal tritium outflow were estimated by the b_n/b_1 ratios and were expressed as percentage of change from the respective mean ratio obtained in the appropriate control; b_n was the fractional rate of outflow in the 5 min period before S_2, S_3 and S_4 (b_2, b_3 and b_4, respectively) and b_1 was the fractional rate of outflow in the 5 min period before S_1. Tritium overflow evoked by electrical stimulation was estimated by subtracting basal outflow from total outflow observed during and in the 10 min period subsequent to each stimulation period, and was expressed as percentage of the total tritium present in the tissue at the onset of stimulation. Effects of drugs added after S_1 on tritium overflow were evaluated as ratios of the overflow elicited by S_2, S_3, S_4, S_5 and S_6 (S_n) and the overflow elicited by S_1 (S_n/S_1). S_n/S_1 values obtained in an experiment in which a test compound "A" was added after S_1 were calculated as a percentage of change (increase or decrease) from the respective mean ratio obtained in the appropriate control group (solvent instead of "A"). When interaction of "A", added after S_1, and a drug "B" either added after S_1 or at the beginning of superfusion, was studied, the "appropriate control" was a group in which B alone was used (Talaia et al., 2005).

4.4. MATERIALS AND SOLUTIONS

The following drugs were used: levo-[ring-2,5,6-^{3}H]-noradrenaline ([^{3}H]-noradrenaline), specific activity 53.0 Ci mmol^{-1} was from DuPont NEN (Garal, Lisboa, Portugal); desipramine hydrochloride, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), yohimbine hydrochloride were from Sigma (Sintra, Portugal). SCH 58261 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine, CGS 21680 2-*p*-(2-Carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine hydrochloride, ANG II acetate Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH.2CH_3COOH.H_2O and SCH 58261 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine. Stock solutions of drugs were prepared with dimethylsulphoxide or distilled water and kept at -20 °C. Solutions of drugs were prepared from aliquots of stock solutions that were diluted in buffer immediately before use (Talaia et al., 2005).

4.5. PRESENTATION OF DATA AND STATISTICAL ANALYSIS

Data are expressed as means ± standard error of the mean (S.E.M.) from n number of tissue preparations. Statistical analysis of the effect of drugs on basal tritium outflow and evoked tritium overflow was carried out using the unpaired Student's t-test or one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. P values lower than 0.05 were considered to indicate significant differences (Talaia et al., 2005).

5. RESULTS

5.1. GENERAL OBSERVATION

In the absence of drugs (except 400 nM desipramine, which was present in the superfusion medium in all experiments to inhibit the neuronal uptake of noradrenaline), the outflow and overflow of tritium was almost constant. The value of S_2/S_1 was 0,98 (n=79). The selective α_2-adrenoceptor antagonist yohimbine (1 μM) when added at the onset of superfusion did not alter basal outflow. Basal outflow and electrically-evoked tritium overflow remained constant throughout experiments, with b_n/b_1 and S_n/S_1 values close to unity (not shown). Drugs added 6, 10, 15 or 25 min before S_2 did not change b_n/b_1 and S_n/S_1 values.

5.2. INFLUENCE OF A_1 BLOCKADE ON EVOKED TRITIUM OVERFLOW

The selective adenosine A_1 receptor antagonist DPCPX was used in these experiments. In all of the experiments DPCPX enhanced the stimulation evoked tritium overflow in a concentration non-dependent manner. Following concentrations were used – 3, 10, 30, 100 and 300 nM. The tissues were stimulated by trains of 2Hz and 200 pulses. The difference between the evoked overflow in artery and vein is shown in fig. 2 and 3. The enhancement of tritium overflow in artery is between 4 %-9 % and in vein 7 %-15 %. For detailed information see table 2.

Fig. 4 and 5 show the influence of the stimulation conditions on the evoked tritium overflow. More specific data are shown in table 3. In the rat mesenteric artery at 2Hz and 200 pulses the facilitation is 15 % ± 5 %, at 8 Hz and 100 pulses 10 % ± 4 % and at 16 Hz and 100 pulses 2 % ± 3 %. In the rat mesenteric vein at 2Hz and 200 pulses 28 % ± 4 %, at 8 Hz and 100 pulses 15 % ± 8 % and at 16 Hz and 100 pulses 33 % ± 5 %.

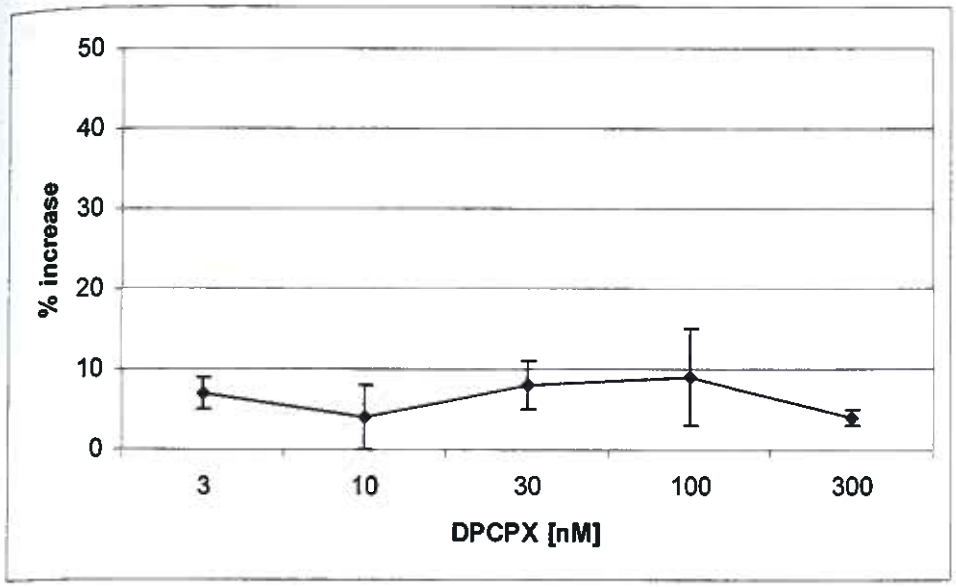

Fig. 2. Effect of DPCPX on evoked tritium overflow in rat mesenteric artery

DPCPX was added 25 minutes before S_n until the end of stimulation period. DPCPX as a potent selective adenosine A_1 receptor antagonist enhances the stimulation evoked tritium overflow. The enhancement is between 4 % and 9 % and as we can see not dependent on the concentration used.

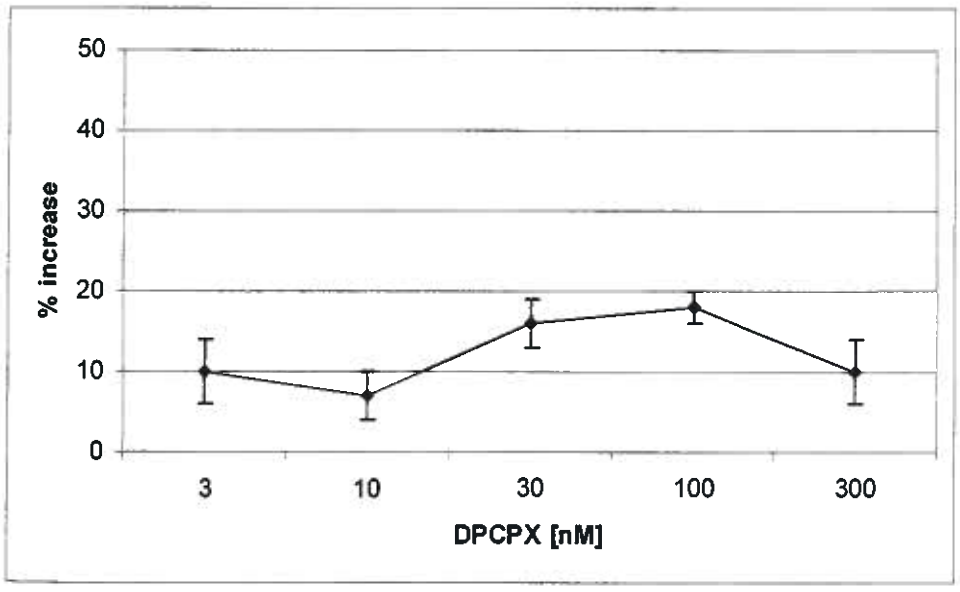

Fig. 3. Effect of DPCPX on evoked tritium overflow in rat mesenteric vein

The stimulation evoked by a selective adenosine A_1 antagonist is between 7 % and 15 % and as we can see not dependent on the used concentration. DPCPX was added 25 minutes before S_n until the end of stimulation period.

Table 2. Complete data of experiments with DPCPX alone

Stimulation conditions	*Concentration [nM]*	*Overflow in artery [%]*	*Overflow in vein [%]*	*Number of experiments*
2Hz-200p	*3*	7±2	10±4	3
	10	4±4	7±3	3
	30	8±3	16±3	3
	100	9±6	18±2	3
	300	4±1	10±4	3

Table 3. Comparing of stimulation conditions - data

Stimulation conditions	*Concentration [nM]*	*Overflow in artery [%]*	*Overflow in vein [%]*	*Number of experiments*
2Hz-200p	100nM	15±5	28±4	6
8Hz-100p	100nM	10±4	15±8	4
16Hz-100p	100nM	2±3	33±5	3

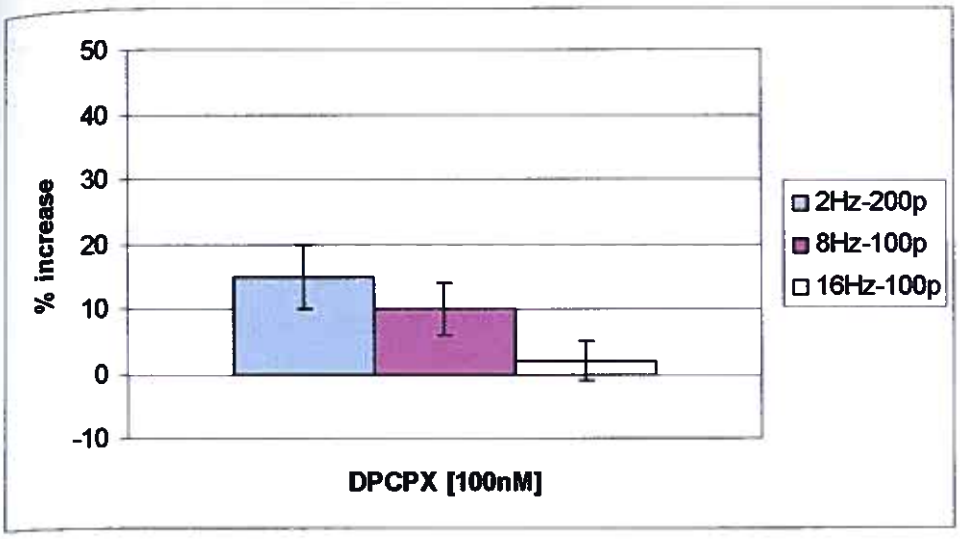

Fig. 4. Effect of DPCPX at different stimulation conditions in artery

DPCPX was added at beginning of superfusion and was present until the end of the experiment. We tried different stimulation conditions changing on one hand the frequency of pulses and on the other hand the number of pulses. This both conditions change the duration of the stimulus group. It seem also to have some influence on evoked tritium overflow.

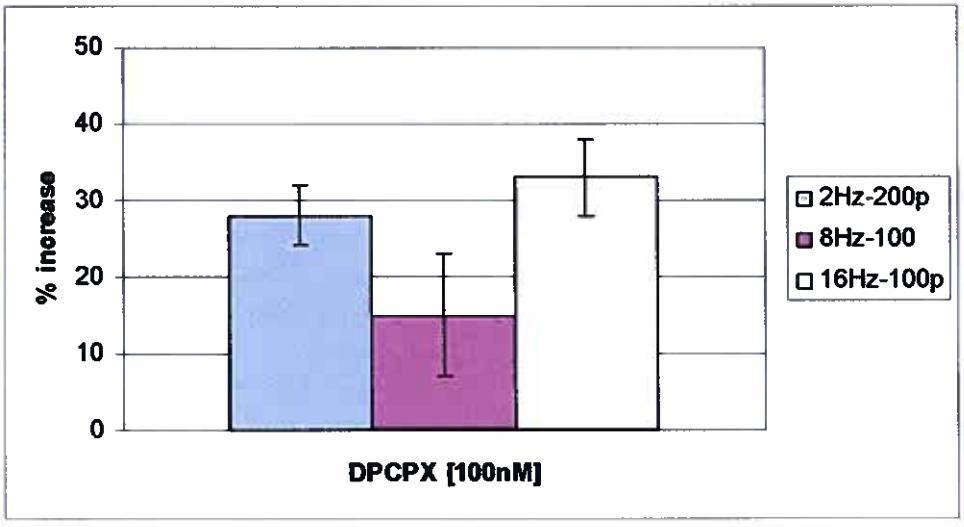

Fig. 5. Effect of DPCPX at different stimulation conditions in vein

DPCPX was added at beginning of superfusion and was present until the end of the experiment. We tried different stimulation conditions changing on one hand the frequency of pulses and on the other hand the number of pulses. This both conditions change the duration of the stimulus group. In case of vein there is no clear influence of the conditions on the evoked tritium overflow.

5.3. INFLUENCE OF A_1 AND α_2 BLOCKADE ON EVOKED TRITIUM OVERFLOW

The selective A_1 antagonist DPCPX and selective α_2 antagonist yohimbine were used in these experiments to verify the role of the influence of presynaptic inhibitory receptors, namely A_1 and α_2. Yohimbine was added at the onset of superfusion and kept throughout the experiment. DPCPX was added 15 min before S_n until the end of stimulation period.

The tritium overflow in the rat mesenteric artery with the concentration of 3 nM was 7 % ± 4 %, with 10 nM 13 % ± 2 %, with 30 nM 7 % ± 3 %, with 100nM 7 % ± 4 % and with 300 nM 12 % ± 4 % (see fig. 6). More concrete data are shown in table 4.

The tritium overflow in the rat mesenteric vein with the concentration of 3 nM was 11 % ± 5 %, with 10 nM 12 % ± 2 %, with 30 nM 11 % ± 3 %, with 100 nM 7 % ± 2 % and with 300 nM 15 % ± 4 % (see fig. 7). More concrete data are shown in table 4.

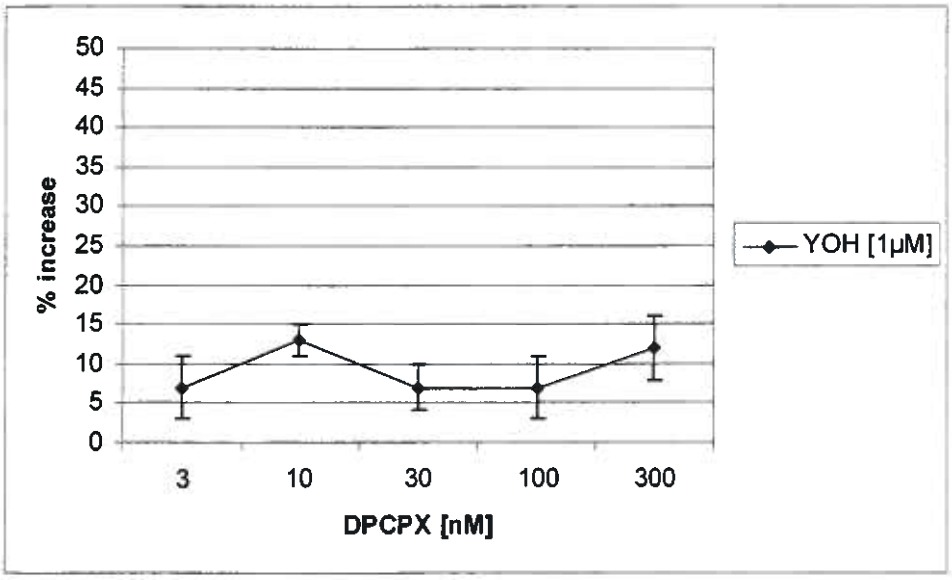

Fig. 6. Effect of DPCPX + yohimbine (YOH) on evoked tritium overflow in rat mesenteric artery

The figure shows the effect when both the receptors A_1 and α_2 are blocked by their selective antagonists. Yohimbine was added at the onset of superfusion and kept throughout the experiment. DPCPX was added 15 min before S_n until the end of stimulation period. The evoked tritium overflow is between 7 % and 13 % and is again concentration non-dependent.

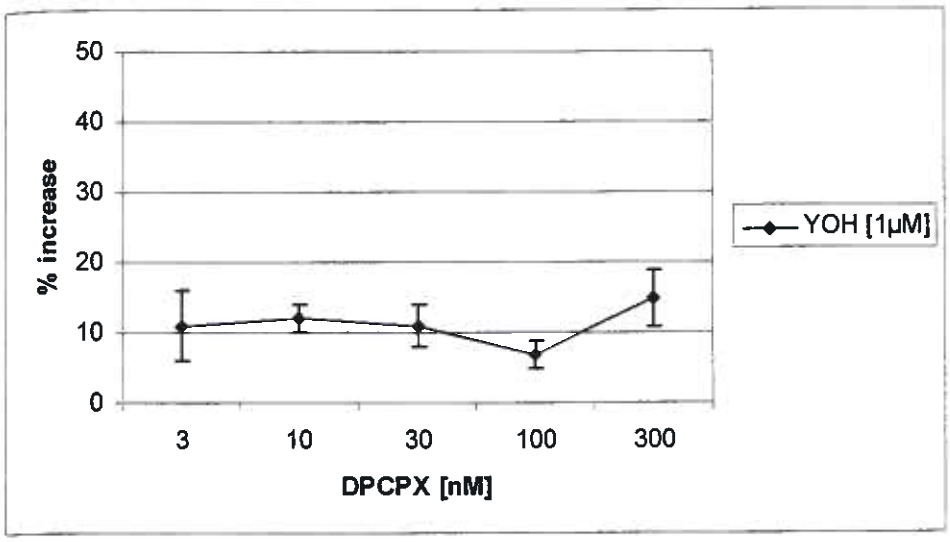

Fig. 7. Effect of DPCPX + yohimbine (YOH) on evoked tritium overflow in rat mesenteric vein

This figure shows how can be the tritium overflow influenced by the blockade of both the receptors A_1 and α_2 by their selective antagonists DPCPX and yohimbine. Yohimbine was added at the onset of superfusion and kept throughout the experiment. DPCPX was added 15 min before S_n until the end of stimulation period. It is easy to see, that when we block more than one autoinhibitory receptors, the increasing effect is much higher than the block of only one of them.

Table 4. Data of experiments with yohimbine and DPCPX together

Stimulation conditions	*Concentration [nM]*	*Overflow in artery [%]*	*Overflow in vein [%]*	*Number of experiments*
2Hz-200p	3	7±4	11±5	9
	10	13±2	12±2	9
	30	7±3	11±3	9
	100	7±4	7±2	9
	300	12±4	15±4	9

5.4. EFFECT OF α_2 RECEPTOR ON EVOKED TRITIUM OVERFLOW

The α_2 receptor is a well known presynaptic autoinhibitory receptor. We used yohimbine as a potent selective antagonist of this receptor. Yohimbine was added 25 minutes before S_n and was present up to the end of the experiments. Especially in these experiments we used a concentration of 3 μM in contrast to the others, where we used 1 μM. Because we wanted to be sure that all of the α_2 receptors are completely blocked. We could find out that α_2 receptors are high effective autonihibitors of noradrenaline release.

The evoked tritium overflow was 626 % ± 83 % in the rat mesenteric artery and 505 % ± 63 % in the rat mesenteric vein. That are the highest diferences between basal outflow and stimulation evoked overflow in all over our experiments. Exact data about the experiments with yohimbine alone can be followed in table 5.

Table 5. Experiments with yohimbine

Stimulation conditions	*Concentration [μM]*	*Overflow in artery [%]*	*Overflow in vein [%]*	*Number of experiments*
2Hz-200p	3	626±83	505±63	6

5.5. EFFECT OF A_{2A} RECEPTORS ON EVOKED TRITIUM OVERFLOW

Adenosine A_{2A} receptors are autoinhibitory receptors expected to modulate the stimulation evoked tritium overflow. To verify the effect of this receptor we used on one hand a selective A_{2A} agonist CGS 21680 and on the other hand a selective antagonist of A_{2A} receptors SCH 58261. The agonist was added 6 minutes before the stimulation and in case of the antagonist 25 minutes before each stimulation to saturate and completely block all A_{2A} receptors. In all of the experiments the concentration we used was 100nM.

The agonist caused in the rat mesenteric artery a decrease of 10 % ± 6 % and the antagonist caused a decrease of 12 % ± 15 %(see fig. 8).

In the rat mesenteric vein the agonist caused a decrease of 20 % ± 8 % and the antagonist an increase of 6 % ± 2 % (see fig. 9).

The results from the arteries are qiute surprising (for detailed data see table 6). The antagonist should increase the stimulation evoked tritium overflow but it decreased.

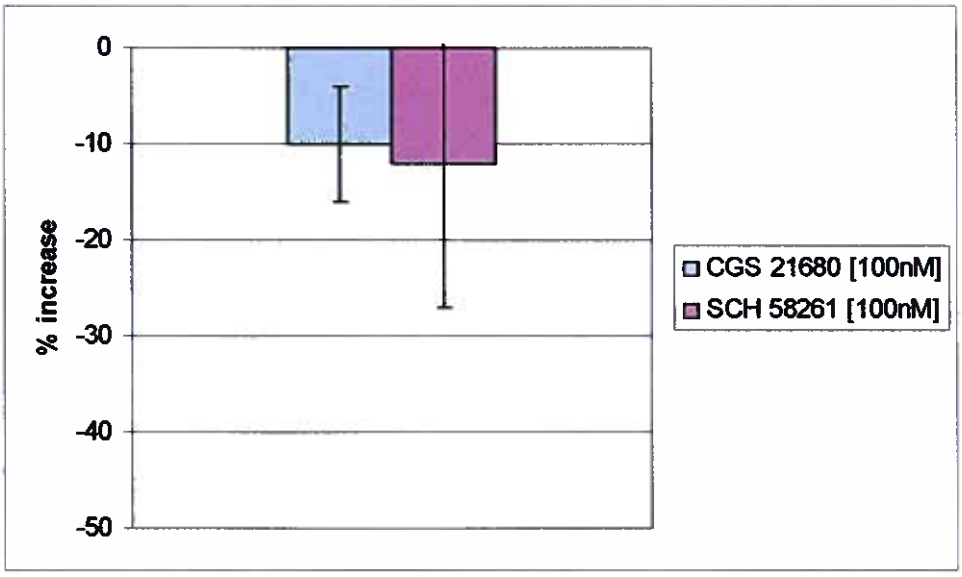

Fig. 8. Effect of CGS 21680 and SCH 58261 on evoked tritium overflow in rat mesenteric artery

The selective A_{2A} receptor agonist CGS 21680 decreased the stimulation evoked tritium overflow and so did the selective A_{2A} receptor antagonist SCH 58261.

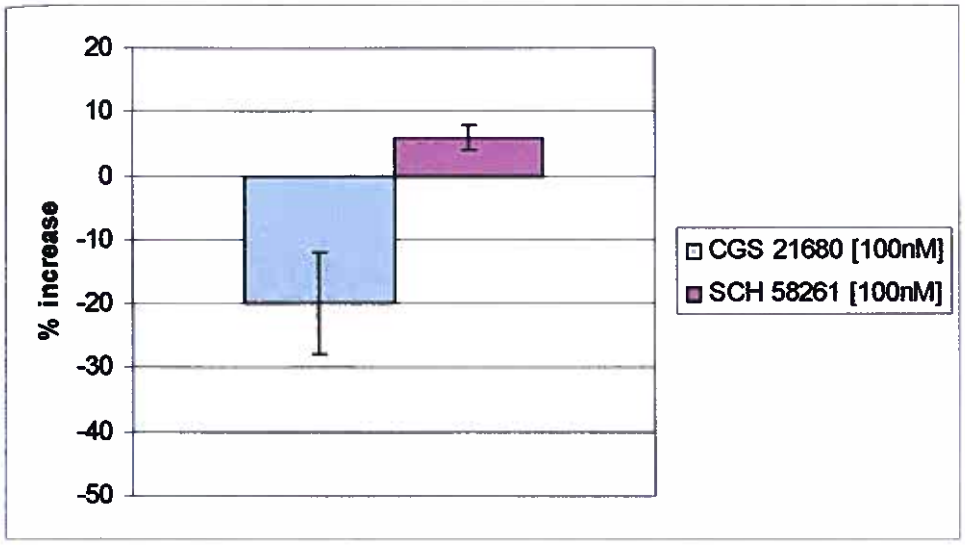

Fig. 9. Effect of CGS 21680 and SCH 58261 on evoked tritium overflow in rat mesenteric vein

The selective A_{2A} receptor agonist CGS 21680 decreased the stimulation evoked tritium overflow. The selective A_{2A} receptor antagonist SCH 58261 gently increased the tritium overflow.

Table 6. Effect of CGS 21680 and SCH 58261

We used on one hand a selective A_{2A} agonist CGS 21680 and on the other hand a selective antagonist of A_{2A} receptors SCH 58261. The agonist was added 6 minutes before the stimulation and in case of the antagonist 25 minutes before each stimulation to saturate and completely block all A_{2A} receptors. In all of the experiments the concentration we used was 100nM.

The agonist caused in the rat mesenteric artery a decrease of 10 % ± 6 % and the antagonist caused a decrease of 12 % ± 15 %. In the rat mesenteric vein the agonist caused a decrease of 20 % ± 8 % and the antagonist an increase of 6 % ± 2 %.

Stimulation conditions	*Drug*	*Concentration [nM]*	*Overflow in artery [%]*	*Overflow in vein [%]*	*Number of experiments*
2Hz-200p	CGS 21680	100	-10±6	-20±8	5
	SCH 58261	100	-12±15	6±2	2

5.6. EFFECT OF AT_1 RECEPTORS ON EVOKED TRITIUM OVERFLOW AT DIFFERENT CONDITIONS

5.6.1. EFFECT OF ANGIOTENSIN II ALONE

AT_1 angiotensin receptors are as presynaptic facilitating receptors involved in modulating of noradrenaline release. In our experiments we used angiotensin II as an agonist. We studied the effect on evoked tritium release of angiotensin II alone, with yohimbine (α_2 receptor antagonist) and with yohimbine and DPCPX (A_1 receptor antagonist) together. The stimulation conditions were constant – 2Hz and 100 pulses. The concentrations of angiotensin were between 0,01 nM and 100 nM. Detailed data see table 7.

The effect of angiotensin II as an agonist at AT_1 receptors seem to be concentration dependent. The stimulation evoked tritium overflow was with 0,01 nM 39 % ± 5 % and 18 % ± 7 % in vein, with 0,1 nM 56 % ± 9 % in artery and 26 % ± 7 % in vein, with 1 nM 89 % ± 7 % in artery and 64 % ± 5 % in vein, with 10 nM 86 % ± 6 % in artery and 69 % ± 7 % in vein and with 100 nM 70 % ± 8 % in artery and 57 % ± 9 % in vein. All these results are well confirmed and not surprising because of our previous knowledge about these receptors.

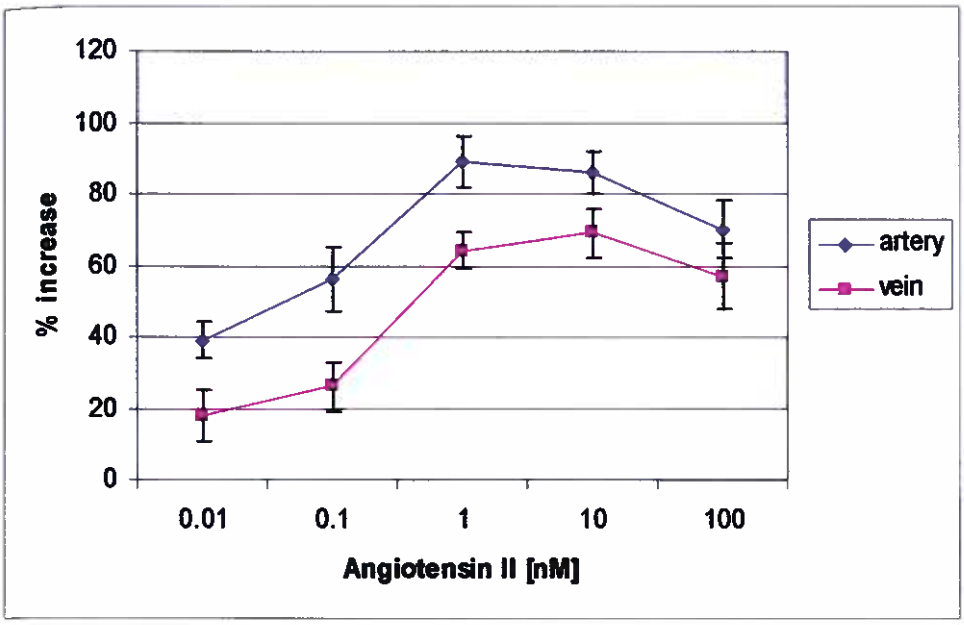

Fig. 10. Effect of angiotensin II on evoked tritium overflow

The effect of angiotensin II as an agonist at AT_1 receptors seem to be concentration dependent. The stimulation evoked tritium overflow was with 0,01 nM 39 % ± 5 % and 18 % ± 7 % in vein, with 0,1 nM 56 % ± 9 % in artery and 26 % ± 7 % in vein, with 1 nM 89 % ± 7 % in artery and 64 % ± 5 % in vein, with 10 nM 86 % ± 6 % in artery and 69 % ± 7 % in vein and with 100 nM 70 % ± 8 % in artery and 57 % ± 9 % in vein.

Table 7. Detailed data of experiments with angiotensin II

Stimulation conditions	*Concentration [nM]*	*Overflow in artery [%]*	*Overflow in vein [%]*	*Number of experiments*
2Hz-100p	0,01	39±5	18±7	6
	0,1	56±9	26±7	6
	1	89±7	64±5	6
	10	86±6	69±7	6
	100	70±8	57±9	6

5.6.2. EFFECT OF ANGIOTENSIN II WITH YOHIMBINE

Angiotensin II is an agonist at AT_1 receptors and yohimbine is an antagonist at α_2 receptors. We studied the influence of both drugs together. So AT_1 receptors are now stimulated and α_2 are blocked. Yohimbine was added at the onset of superfusion and kept throughout the experiment. Angiotensin was added 6 minutes before each stimulation and was present to the end of the stimulation period. The concentration of yohimbine was 1 μM and the concentration of angiotensin II was between 0,01 nM and 100nM. The tissues were stimulated by trains of 100 pulses at 2Hz.

The tritium overflow in rat mesenteric artery changed like this: 0,01 nM 1 % ± 5 %, with 0,1 nM 12 % ± 6 %, with 1 nM 27 % ± 8 %, with 10 nM 28 % ± 7 % and with 100 nM 21 % ± 8 % (see fig. 11).

The tritium overflow in rat mesenteric vein changed like this: with 0,01 nM 1 % ± 3 %, with 0,1 nM 10 % ± 4 %, with 1 nM 16 % ± 4 %, with 10 nM 24 % ± 6 % and with 100 nM 19 % ± 6 % (see fig. 12). For completely detailed data see table 8.

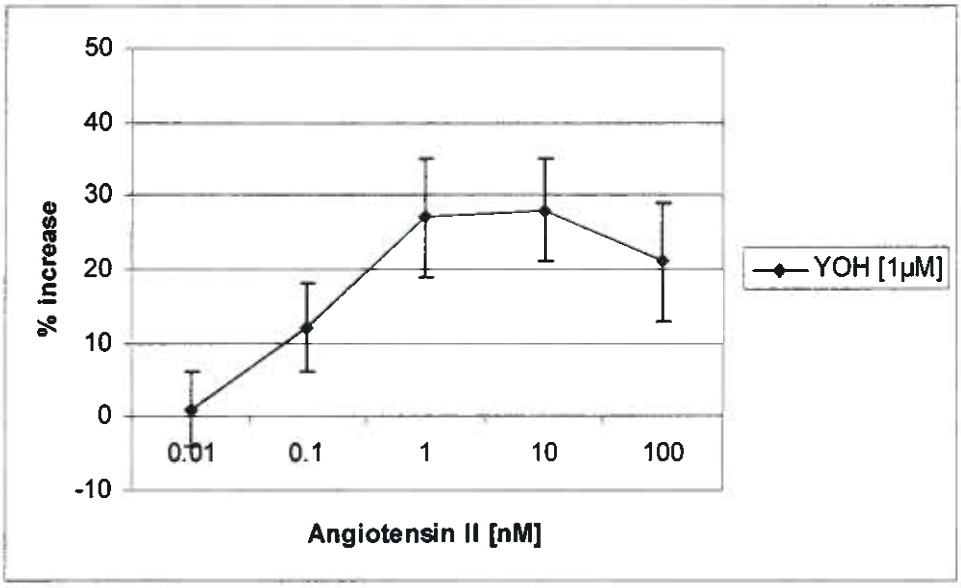

Fig. 11. Effect of angiotensin II and yohimbine on evoked tritium overflow in artery

Angiotensin II is an agonist at AT_1 receptors and yohimbine is an antagonist at α_2 receptors. Yohimbine was added at the onset of superfusion and kept throughout the experiment. Angiotensin was added 6 minutes before each stimulation and was present to the end of the stimulation period.

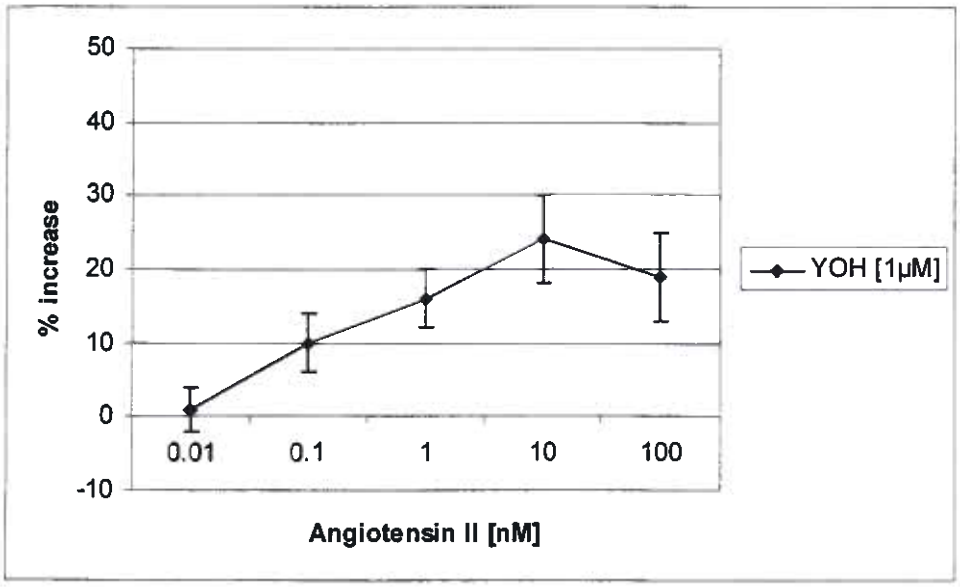

Fig. 12. Effect of angiotensin II and yohimbine on evoked tritium overflow in vein

Angiotensin II is an agonist at AT_1 receptors and yohimbine is an antagonist at α_2 receptors. Yohimbine was added at the onset of superfusion and kept throughout the experiment. Angiotensin was added 6 minutes before each stimulation and was present to the end of the stimulation period. The tritium overflow in rat mesenteric vein changed like this: with 0,01 nM 1 % ± 3 %, with 0,1 nM 10 % ± 4 %, with 1 nM 16 % ± 4 %, with 10 nM 24 % ± 6 % and with 100 nM 19 % ± 6 %.

Table 8. Effect of angiotensin II and yohimbine on evoked tritium overflow in rat mesenteric artery and vein

Stimulation conditions	*Concentration*		*Overflow in artery [%]*	*Overflow in vein [%]*	*Number of experiments*
	YOH[μM]	*ANG II [nM]*			
2Hz-100p	1	0,01	1±5	1±3	5
	1	0,1	12±6	10±4	5
	1	1	27±8	16±4	5
	1	10	28±7	24±6	5
	1	100	21±8	19±6	5

5.6.3. EFFECT OF ANGIOTENSIN II WITH α_2 BLOCKED BY STIMULATION CONDITIONS

We studied the influence on the stimulation evoked tritium overflow with stimulating conditions simulating α_2 blockade. We stimulated the tissues with trains of 50 Hz and only 20 pulses. The stimulation group duration is only 400 ms and therefore the autoinhibitory α_2 receptors are out of influence because it is to short duration to involve them in modulation of noradrenaline release. We expected similar results to the experiments with angiotensin and yohimbine together. Angiotensin II was added 6 minutes before each stimulation and was present to the end of it.

As you can see, the stimulation conditions completely abolished the previous effect of angiotensin II on the tritium overflow (fig. 13 and 14). The increase of tritium overflow caused by angiotensin II in artery was: with 0,01 nM 2 % ± 7 %, with 0,1 nM -3 % ± 7 %, with 1 nM 2 % ± 5 %, with 10 nM 3 % ± 5 % and with 100 nM 2 % ± 6 % (see fig. 12).

The increase of tritium overflow caused by angiotensin II in vein was: with 0,01 nM 0 % ± 7 %, with 0,1 nM 2 % ± 7 %, with 1 nM 7 % ± 9 %, with 10 nM 0 % ± 9 % and with 100 nM -6 % ± 10 % (see fig. 13). For more data see table 9.

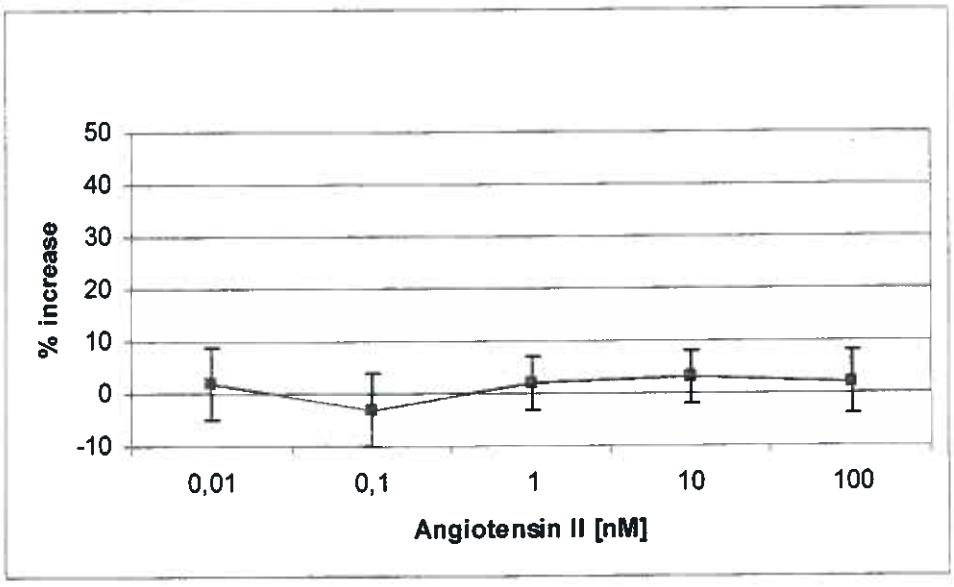

Fig. 13. Effect of angiotensin II on the evoked tritium overflow in rat mesenteric artery at 50 Hz-20pulses (simulation of α_2 block)

The previous stimulatory effect of angiotensin II is completely abolished by the stimulation conditions.

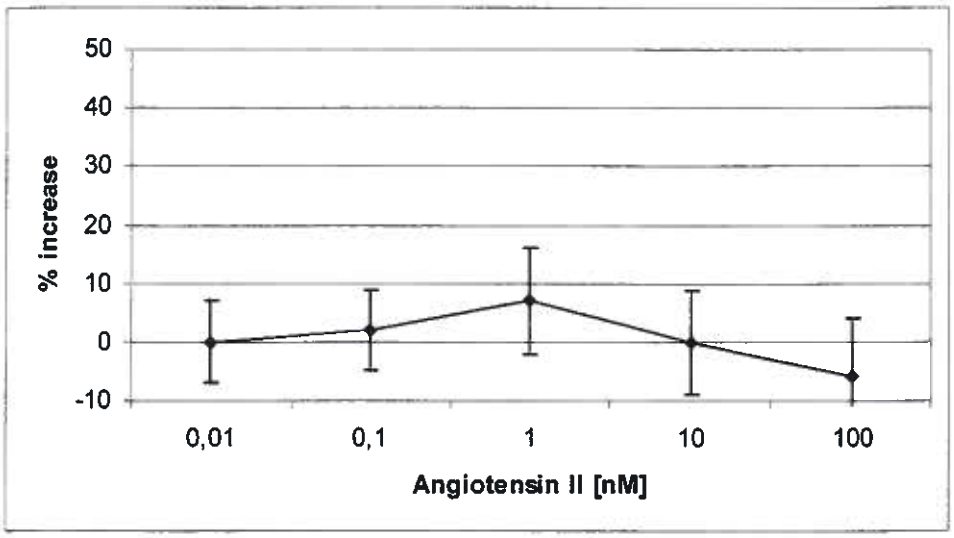

Fig. 14. Effect of angiotensin II on the evoked tritium overflow in rat mesenteric vein at 50 Hz-20 pulses (simulation of α_2 block)

The previous stimulatory effect of angiotensin II is completely abolished by the stimulation conditions.

Table 9. Detailed data about the effect of angiotensin II at 50 Hz-20p (simulation of α_2 block)

Stimulation conditions	*Concentration [nM]*	*Overflow in artery [%]*	*Overflow in vein [%]*	*Number of experiments*	
				artery	vein
50Hz-20p	0,01	2±7	0±7	9	6
	0,1	-3±7	2±7	9	5
	1	2±5	7±9	8	5
	10	3±5	0±9	9	6
	100	2±6	-6±10	9	5

5.6.4. EFFECT OF ANGIOTENSIN II WITH YOHIMBINE AND DPCPX

We were curious about the influence of the combination of A_1 and α_2 receptor antagonists with the AT_1 receptor agonist angiotensin II. Yohimbine and DPCPX were added at the onset of superfusion and kept throughout the experiment. Angiotensin was added 6 minutes before each stimulation. The stimulation was made by trains of 2 Hz and 100 pulses. The concentration of the drugs was following: DPCPX 100 nM, yohimbine 1 μM and angiotensin II 10 nM. Under these conditions we obtained next results (see fig. 15).

This figure below shows the modulatory effect of AT_1 receptors when the A_1 and α_2 are blocked. The increase of stimulation evoked tritium overflow was in artery 37 % ± 3 % and in vein 14 % ± 3%. We did 8 experiments with artery and with vein so these results are representative.

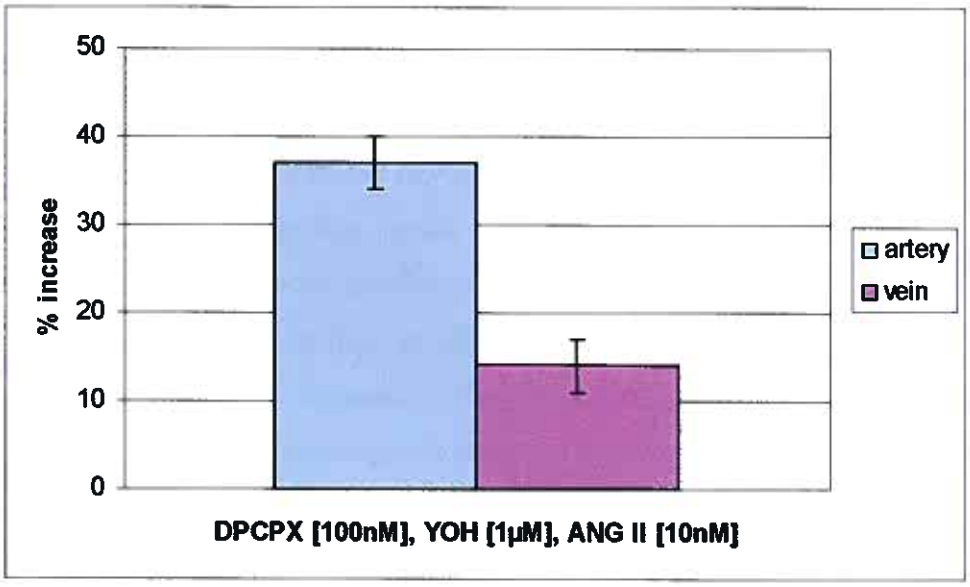

Fig. 15. Effect of angiotensin II with yohimbine and DPCPX in rat mesenteric artery and vein

This figure shows the modulatory effect of AT_1 receptors when the A_1 and α_2 are blocked. The increase of stimulation evoked tritium overflow was in artery 37 % ± 3 % and in vein 14 % ± 3%.

6. DISCUSSION

Tritium overflow, evoked by electrically stimulation of tissue preparations of rat mesenteric vessels pre-incubated with [^{3}H] noradrenaline, was assumed to mimic the action potential-evoked neuronal noradrenaline release. We supposed an inhibitory effect of α_2, A_1 and A_{2A} receptors on stimulation evoked tritium overflow. On the other side we supposed a facilitatory effect of the AT_1 receptors.

Adenosine A_1 receptors couple to G proteins of the $G_{i/o}$ class. Upon reconstitution, the receptor clearly prefers G_i to G_o (Klinger et. al, 2001). We used a high potent selective A_1 receptor antagonist DPCPX to modulate the stimulation evoked noradrenaline release. We supposed that when we block A_1 receptors as autoinhibitory presynaptic receptors we increase the noradrenaline release. We could verify our presumption because the block of the adenosine A_1 receptor increased the stimulation evoked tritium overflow. The increase was between 4 – 9 % in artery and 7 -15 % in vein (see fig. 2 and 3).

We were curious if an additional block of α_2 receptors is of influece on evoked tritium overflow. We used a selective α_2 antagonist yohimbine to block this receptors in a concentration previously confirmed to block the receptors. We expected more facilitatory effect than with DPCPX alone. Our presumption was again well-founded because the increase of noradrenaline release was similar to experiments with DPCPX alone. The values ranged between 7 – 13 % in artery and between 7 – 15 % in vein (see fig. 6 and 7). The results show us that the effect of A_1 and α_2 are not additional. But why is the effect of the drugs together not additional? A possible reason is that the A_1 and α_2 receptors couple to the same G protein family. The A_1 receptors couple as mentioned above to the $G_{i/o}$ family. But the α_2 receptors can couple not only to pertussis toxin substrates (G_i and G_o), as as it does in physiological settings, but also to activation of phospholipase C, presumably through interaction with $G_{q/11}$, and to stimulation of adenylyl cyclase through G_s (Chabre et al., 1994).

Furthermore, we studied the effect of A_{2A} adenosine receptors on the evoked tritium release. CGS 21680, a selective A_{2A} receptor agonist, and SCH 58261, a selective A_{2A} receptor antagonist, were used in these experiments. The results were very strange and surprising. Neither CGS 21680 nor SCH 58261 significantly changed the stimulation evoked tritium overflow. There was only a small decreasing effect caused by CGS 21680 on the

evoked tritium overflow. All-around these experiments with the A_{2A} receptor agonist and antagonist did not play any significant role in modulating the noradrenaline release. In some previous papers CGS 21680 caused a significant increase of the tritium overflow by the stimulation by 5 Hz and 100 pulses but no significant effect by trains of 50 Hz and 20 pulses (Fresco et al., 2002). Our stimulating conditions (2Hz and 200 pulses) were different from their stimulating conditions. But I am not sure if only the stimulation conditions are the main reason for this disagreement.

In the next part of our experiments we studied the effect of α_2 receptors. We used a selective α_2 receptor antagonist yohimbine to verify the increasing effect of α_2 blocked receptors on evoked tritium overflow. This drug caused a very high increase of evoked noradrenaline release. The evoked tritium overflow was approximately 6 fold higher in the rat mesenteric artery and 5 fold higher in the rat mesenteric vein than the basal outflow of tritium. These results showed us what a big influence do the α_2 presynaptic autoinhibitory receptors have on modulating noradrenaline release.

A big number of our experiments we aimed on the angiotensin II AT_1 receptors. We compared the modulatory effect of angiotensin II on stimulation evoked tritium overflow under different stimulatory conditions. First we verified the stimulatory effect of angiotensin II alone. Than we tried under α_2-autoinhibition rich conditions (2 Hz and 200 pulses) the effect of angiotensin II with pharmacologically blocked α_2 receptors and finally we studied the effect of angiotensin II under α_2-autoinhibition poor conditions (50 Hz and 20 pulses).

Presynaptic angiotensin receptors couple to $G_{q/11}$ proteins. The presynaptic angiotensin II receptors are discovered to be AT_1 because the facilitatory effect of angiotensin II was blocked respectively by the AT_1 receptor antagonist losartan (Cox et al., 2000). Our experiments showed that a stimulation of presynaptic AT_1 receptors causes an increase of stimulation evoked noadrenaline release (see fig. 10). But this increase was attenuated by usage of α_2 receptor antagonist yohimbine (see fig. 11 and 12). Finally we tried to stimulate under poor α_2 autoinhibition conditions. We used trains of 50 Hz and 20 pulses. The effect of angiotensin II was completely abolished (see fig. 13 and 14). Similar results have been published few years ago by Cox et al.(2000) and Trendelenbutg et al. (2003). They are suggesting following explanation of such interesting effects. Angiotensin II increases the release of noradrenaline at least to a large extent by interrupting an ongoing α_2-autoinhibition. More specifically, they suggested that one effector at AT_1 receptors, namely protein kinase C, inactivates a protein involved in the presynaptic α_2-autoreceptor $\rightarrow$ $G_{i/o}$ $\rightarrow$ presynaptic calcium channel inhibitory pathway. In support of this view, when α_2-autoinhibition was

absent, activation of other $G_{i/o}$-coupled presynaptic receptors, namely neuropeptide Y Y_2- and opioid OP_1-receptors, enabled angiotensin II to produce its full scope of facilitation. It has been shown a number of years ago, that angiotensin II caused a greater increase in noradrenaline release in rabbit atria when presynaptic muscarinic receptors were activated simultaneously. Perhaps, this was because of an analogous mechanism, that is in this case muscarinic stimulation of the presynaptic $G_{i/o}$ pathway, thus giving angiotensin II greater space for disinhibition (Trendelenburg et al., 2003). I agree to this hypothesis.

But we were still curious about the effect of angiotensin II when α_2 and A_1 receptors are pharmacologically blocked. To block the A_1 receptors we used DPCPX and to block the α_2 receptors we used yohimbine. The results showed us that the blockade of A_1 receptors gently restored the facilitatory effect of angiotensin II (see fig. 15). This could be either because of the facilitatory effect of A_1 receptor blockade alone or because of any intaraction in the transduction pathways. I personally believe in the first hypothesis. And that is due to the effect of DPCPX alone on evoked tritium overflow which was similar to this restoring effect of noradrenaline release (see fig. 2 and 3).

7. SUMMARY

General pharmacological characteristics of α_2 and of adenosine receptors and especially of adenosine A_1 and A_{2A} receptor were briefly mentioned in the first, theoretical part of this work. G-protein coupling, signal transduction mechanism and interactions with other receptor systems were explained. The mechanism(s) of the interaction occurring between adenosine AT_1 receptors and α_2-adrenoceptors were studied in this work by elucidating the signalling events triggered by co-activation of these two G protein-coupled receptors. Tritium overflow, evoked by electrically stimulation of tissue preparations of rat mesenteric vessels pre-incubated with [^{3}H] noradrenaline, was assumed to mimic the action potential-evoked neuronal noradrenaline release. Drug-induced changes in the evoked tritium overflow were assumed to reflect changes in neuronal release of noradrenaline during absence or presence of drugs.

The block of A_1 receptors by a selective antagonist DPCPX caused an increase of tritium overflow and this effect was further developed by additional block of the presynaptic autoinhibitory α_2 receptors caused by yohimbine.

None of the experiments with adenosine A_{2A} receptors showed interesting and usefull results. We used CGS 21680 as a selective agonist and SCH 58261 as a selective antagonist of A_{2A} receptors. But neither the stimulation nor the block of adenosine A_{2A} receptors modulated the stimulation evoked overflow in a significant manner.

Finally we focused our research on interactions between angiotensin AT_1 and α_2 adrenoceptors. The stimulation of AT_1 receptors caused by angiotensin II triggered an increase of tritium overflow. But under conditions of poor ongoing α_2-autoinhibition (50 Hz and 20 pulses) the effect was abolished. Under conditions of high ongoing α_2-autoinhibition (2Hz and 200 pulses) the block of α_2 receptors greatly attenuated the effect of angiotensin II respectivelly. This could be due to the fact that angiotensin II increases the release of noradrenaline at least to a large extent by interrupting an ongoing α_2-autoinhibition. More specifically, it was suggested that one effector at AT_1 receptors, namely protein kinase C, inactivates a protein involved in the presynaptic α_2-autoreceptor $\rightarrow$ $G_{i/o}$ $\rightarrow$ presynaptic calcium channel inhibitory pathway. Then we studied the effect of A_1 and α_2 block on evoked tritium overflow caused by angiotensin II. When A_1 and α_2 blocked we found out that the

blocked A_1 receptors gently restore the effect of angiotensin II. But i suppose this is due to the own effect on evoked tritium overflow caused by the selective A_1 receptor antagonist DPCPX.

8. SOUHRN

Základní farmakologická charakteristika adrenergních α_2 a adenosinových receptorů především A_1 a A_{2A} je podrobně rozebrána v první teoretické části této práce. Bylo vysvětleno napojení na jednotlivé G proteiny, dány příklady agonistů i antagonistů a uvedeny obecné mechanismy interakcí mezi receptory. Tato práce je zaměřena na studium interakcí mezi presynaptickými angiotensinovými AT_1 receptory a adrenergními α_2 receptory. Simulace fyziologického uvolnění noradrenalinu probíhala pomocí elektrických impulsů, kde se následně z tkání (mesenterické cévy potkana), které byly předem inkubovány s radioaktivně označeným noradrenalinem, uvolnil noradrenalin. Přítomnost či nepřítomnost různých farmak měla stimulovat či inhibovat uvolnění noradrenalinu ze tkání.

Blokáda adenosinových A_1 receptorů pomocí selektivního antagonisty DPCPX způsobila zvýšení uvolnění noradrenalinu. Tento efekt byl ještě zesílen pomocí blokády adrenergních α_2 receptorů pomocí selektivního antagonisty yohimbinu.

Kupodivu experimenty a adenosinovými A_{2A} receptory nepřinesly žádné zajímavé ani očekávané výsledky. Použití CGS 21680 (selektivní agonista) ani SCH 58261 (selektivní antagonista) nezpůsobilo výraznou změnu v uvolnění noradrenalinu, která by stála za zmínku a komentář. Vysvětlení tohoto nálezu by mohlo spočívat v odlišnostech použitých potkanů, které pocházely ze dvou různých zdrojů. K těmto odchylkám docházelo totiž v této pracovní skupině již v minulosti.

Zaměřili jsme se proto na studium interakcí mezi angiotensinovými AT_1 receptory a adrenergními α_2 receptory. Stimulace AT_1 receptorů měla za následek mohutné zvýšení uvolnění noradrenalinu. Toto bylo ovšem kompletně zrušeno za podmínek, které neumožňují autoinhibici adrenergních presynaptických α_2 receptorů. Proto jsme pokus opakovali za normálních (α_2 autoinhibičních) podmínek, ovšem v přítomnosti α_2 blokátoru yohimbinu. Yohimbin sice výrazně snížil původní vysoký efekt angiotensinu, ale nedošlo k úplnému vyrušení jako v předchozím případě. Toto se dá vysvětlit tak, že angiotensin zvyšuje uvolnění noradrenalinu pomocí přerušení inhibičního vlivu adrenergních presynaptických autoinhibičních α_2 receptorů. Jeden člen z přenosu signálu od receptoru k efektoru, konkrétně protein kináza C, inaktivuje jiný protein zapojený do přenosu signálu od adrenergních α_2

receptorů a tím jejich inhibiční vliv sníží. Toto je hlavním a nejdůležitějším výsledkem naší práce.

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