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Characterization of α_1-adrenoceptor subtypes in the pig

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Abstract

The identities of the α_1-adrenoceptor subtypes present in various tissues of the pig were studied using [^{3}H]prazosin radioligand binding. The subtypes were characterized by performing competition experiments for various subtype selective drugs. In the cerebral cortex, spleen and heart, both α_{1A}- and α_{1B}-adrenoceptors were detected. In the liver was found only the α_{1A}-subtype, while in the aorta was found only the α_{1B}-subtype. An α_1-adrenoceptor subtype was present in the adrenal gland with a high affinity for prazosin, the pK_d value being 9.6, but with relatively low affinities for other α_1-adrenoceptor binding drugs. The adrenal gland α_1-adrenoceptor did not seem to represent the classical α_{1D}-subtype, since drugs selective for the α_{1D}-subtype in other species, including BMY7378 and SKF104856, showed low affinities for the pig adrenal gland α_1-adrenoceptor. © 1998 Elsevier Science B.V.

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1. Introduction

Three α_1-adrenoceptor subtypes have been cloned, and pharmacologically three corresponding native α_1-adrenoceptors have been identified. In accordance with an IUPHAR report (Bylund et al., 1994; Hieble et al., 1995), the native α_1-subtypes are currently defined as α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (upper case), while the corresponding cloned subtypes are denoted α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors (lower case). The α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes have been cloned from several species, including rat and human (see the works of Ford et al. (1994), Hieble et al. (1995), Michel et al. (1995) and Guarino et al. (1996)). To date, however, none of the porcine α_1-adrenoceptor subtypes have been cloned.

Homogenous populations of α_{1A}-adrenoceptors have been reported to be present in the rat submaxillary gland, the rabbit liver and the human prostate and liver. It coexists with other subtypes in the rat cerebral cortex, hippocampus, vas deferens, kidney and heart (Goetz et al., 1994; Michel et al., 1995; Hieble et al., 1995). Homogenous populations of α_{1B}-adrenoceptors have been found in the rat liver and spleen (Hieble et al., 1995). It coexists with other α_1-adrenoceptor subtypes in the rat cerebral cortex, hippocampus, kidney and heart (Michel et al., 1995). Binding and physiological studies indicate that the α_{1D}-adrenoceptor is present in the rat aorta. (Kenny et al., 1995; Testa et al., 1995; Deng et al., 1996; Saussy et al., 1996). The level of α_{1d} mRNA has been reported to be high in the rat aorta, adrenal gland, vas deferens and cerebral cortex (Piascik et al., 1994; Scofield et al., 1995) and in the human aorta and cerebral cortex (Price et al., 1993).

Substances with selectivity for the α_{1A}- over the α_{1B}- and α_{1D}-subtypes include WB4101, 5-methyl-urapidil, niguldipine and oxymetazoline. Spiperone and risperidone have been shown to be selective for the α_{1B}- over the α_{1A}-subtype in the rat (Michel et al., 1989; Sleight et al., 1993). The compound BMY7378 has been reported to be α_{1D}-selective, with a pK_i value in the range 8.2 to 9.4 for α_{1D}-adrenoceptors in rat and human (Kenny et al., 1995; Deng et al., 1996; Saussy et al., 1996). Another substance that has been reported to be α_{1D}-selective is SKF104856 (Ruffolo et al., 1995).

All three α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors bind prazosin with high affinity (pK_i values > 9.0). However, in

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addition to these well-defined α_1-adrenoceptors, a subpopulation of α_1-adrenoceptors with low affinity for prazosin, denoted α_{1L} (pK_i value < 9.0), has been proposed to exist in vascular tissues (Muramatsu et al., 1990; Ohmura et al., 1992; Oshita et al., 1993). However, to date no gene corresponding to the α_{1L}-adrenoceptor has been cloned, and it has even been proposed that the receptor represents a particular conformational state of the α_{1A}-adrenoceptor subtype (Ford et al., 1997).

In the present paper, we have characterized the α_1-adrenoceptor subtypes present in pig tissue membranes on the basis of the criteria defined in the IUPHAR α_1-adrenoceptor subclassification (Hieble et al., 1995). The aim was to characterize pig α_1-adrenoceptor subtypes, and to identify which standard α_1-subtype selective compounds that are selective between the α_1-adrenoceptor subtypes in the pig. Receptors resembling α_{1A}- and α_{1B}-adrenoceptors were detected in various tissues. In addition, an undefined α_1-adrenoceptor subtype was detected in the adrenal gland. Oddly, WB4101 showed higher affinity for α_{1A}-adrenoceptors from cerebral cortex than from liver. The results represent the first broad pharmacological characterization of the pig α_1-adrenoceptor subtypes.

2. Material and methods

2.1. Membrane preparations

Pig tissues from the liver, spleen, adrenal gland, cerebral cortex, aorta and heart were obtained from a local slaughterhouse. The excised tissues were immediately placed on ice, cut into smaller pieces and frozen at -80°C within 1 h. Membranes were prepared from thawed samples essentially as described previously (Uhlén and Wikberg, 1991a). The final pellets were resuspended in 1.5 mM EDTA, 50 mM Tris–HCl, at pH 7.5 with protein concentrations of about 2.0 mg/ml for liver, 1.4 mg/ml for spleen, 2.0 mg/ml for adrenal gland, 1.2 mg/ml for cerebral cortex, 0.8 mg/ml for aorta and finally 2.8 mg/ml for the heart. Protein concentrations were measured according to Lowry et al. (1951).

2.2. Binding studies

Radioligand binding was performed as described (Uhlén and Wikberg, 1991a) by incubating 80–280 μg of membrane protein in 150 μl of 100 μM Gpp(NH)p (guanyl-5′-yl-imido-diphosphate), 140 mM NaCl, 33 mM Tris–Cl, at pH 7.5 with [^{3}H]prazosin and drugs for 1 h at 25°C and then filtering and washing on Whatman GF/C filters. All assays were performed in duplicate. The figures and the correlation analysis were constructed using DeltaGraph Pro 3.5 from DeltaPoint.

Computer modelling of the data was performed as described by Uhlén and Wikberg (1991b), using a radioligand binding analysis package (Wan system, Umeå, Sweden) on a MacIntosh computer. It can be noticed that in the computer analysis of the binding data, it is assumed that each ligand binds to each of the receptors according to the law of mass action. For example, in the competition experiments in the cerebral cortex, the binding of the

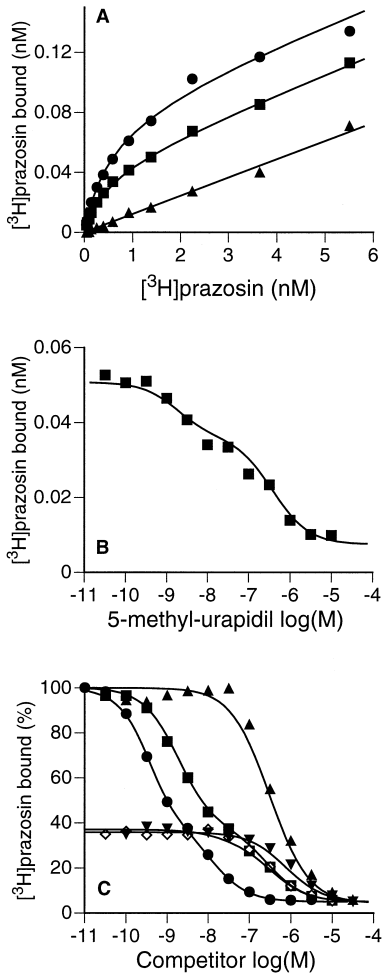

Fig. 1. (A,B) Combined [^{3}H]prazosin saturation curves and competition curve for 5-methyl-urapidil performed on pig cerebral cortex membranes. The saturation curves shown in (A) represent total [^{3}H]prazosin binding (●), binding of [^{3}H]prazosin in the presence of 60 nM 5-methyl-urapidil (■) and binding in the presence of 20 μM WB4101 (▲). Shown in (B) is the competition curve of 5-methyl-urapidil (■) obtained in the presence of 0.6 nM [^{3}H]prazosin, using the same membranes and performed on the same time as the experiment shown in panel A. Panels A and B show one representative experiment out of three. (C) Competition curves for drugs competing for [^{3}H]prazosin binding to α_{1A}- and α_{1B}-adrenoceptors. Pig cerebral cortex membranes were incubated with 0.6 nM [^{3}H]prazosin and various concentrations of WB4101 (●), 5-methyl-urapidil (■), BMY7378 (▲), 5-methyl-urapidil in the presence of 3 nM WB4101 (◇) and BMY7378 in the presence of 3 nM WB4101 (▼).

radioligand B_r to each receptor is described by the formula:

$$B_r = \frac{B_{max}[C_r]}{K_{dr} + (K_{dr}/K_{dw})[C_w] + (K_{dr}/K_{dc})[C_c] + [C_r]}.$$

The variables $[C_r]$, $[C_w]$ and $[C_c]$ are the concentrations of the radioligand, WB4101 and competitor, respectively, and the constants K_{dr}, K_{dw} and K_{dc} are the K_d values of the radioligand, WB4101 and competitor, respectively. Thus, the influence by each drug present in the assay is taken into account in the computer analysis of the binding data. In tissues where two receptors were labelled, the formula was expanded to account for two receptors (two-site model). The free concentrations of drugs, e.g., the radioligand $[C_r]$, were calculated iteratively by formulas of the form $[C_r] = [C_r]_{total} - B_r$ − non-specific binding. Since the non-specific binding only for the radioligand is directly detected, the fractional non-specific binding of competing drugs were assumed to be equal to that of the radioligand.

The combined saturation and competition experiments in cerebral cortex membranes were performed using a multi-curve approach (Uhlén and Wikberg, 1991b). This experimental design is exemplified in Fig. 1A–B, which shows the results from the simultaneous fitting of all four curves of one experiment (shown in Fig. 1A–B) to a two-site model. The reason for including 60 nM of 5-methyl-urapidil in one of the saturation curves (Fig. 1A) was because this concentration of 5-methyl-urapidil blocks the majority of the α_{1A}-adrenoceptors, while leaving most of the α_{1B}-adrenoceptors unblocked. The inclusion of a competition curve of 5-methyl-urapidil helps in defining the proportions of the α_{1A}- and α_{1B}-adrenoceptors in each experiment. Thereby, the pK_i values of [^{3}H]prazosin for both α_{1A}- and α_{1B}-adrenoceptors could be determined simultaneously. Non-specific binding was defined by a saturation curve in the presence of 20 μM of WB4101.

In the competition experiments performed in cerebral cortex membranes a competition curve for the tested drug, a competition curve for WB4101, and a competition curve for the tested drug in the presence of 3 nM of WB4101 were obtained simultaneously. Around 3 nM of WB4101 is blocking predominantly the α_{1A}-subtype, leaving the α_{1B}-subtype almost unaffected. For example, at 0.6 nM of [^{3}H]prazosin, 3 nM of WB4101 and low concentrations of the varying competitor (exemplified by the binding at the intercept on the *y*-axis in the WB4101 blocked curves in Fig. 1C), the radioligand labels about 63% of the total amount of α_{1B}-adrenoceptors present in the assay, and only about 2.4% of the α_{1A}-adrenoceptors. The reason for using WB4101 as the discriminative α_{1A}-selective drug in some sets of experiments, and 5-methyl-urapidil in others, was that the more selective compound 5-methyl-urapidil was not available from the purchaser at the beginning of our study.

2.3. Isotopes, drugs and chemicals

[7-methoxy-^{3}H]prazosin (74 Ci/mmol) was from New England Nuclear (NEN) through DuMedical, Stockholm, Sweden. BMY7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione), methiothepin, 5-methyl-urapidil, *S*(+)-niguldipine, risperidone, spiperone and WB4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane) were from Research Biochemicals, Natick, MA. Chlorpromazine, (−)-epinephrine, (−)-norepinephrine and prazosin were from Sigma, St. Louis, MO. Clozapine and thioridazine were gifts from Sandoz, Basel, Switzerland. *S*(+)-Niguldipine; (+)-epinephrine and (+)-norepinephrine were gifts from Sterling-Winthrop, Rensselaer, NY. Oxymetazoline was a gift from Draco, Lund, Sweden. SKF104856 was a gift from Smith, Kline and French, Swedeland, PA. GppNHp was from Boehringer/Mannheim, Mannheim, Germany.

3. Results

3.1. Determination of pK_d values of [^{3}H]prazosin for α_{1A}- and α_{1B}-adrenoceptors in the pig cerebral cortex

In pilot experiments using [^{3}H]prazosin as radioligand, competition curves were obtained for WB4101 and 5-methyl-urapidil in pig cerebral cortex membranes. This was done in order to probe what α_1-adrenoceptor subtypes were present in this tissue. Both WB4101 and 5-methyl-urapidil have been shown in other species to be highly α_{1A}- vs. α_{1B}-selective, as well as slightly α_{1A}- vs. α_{1D}-selective (Michel et al., 1995). The resulting curves were clearly biphasic, indicating that the pig cerebral cortex contained two types of α_1-adrenoceptors, which we interpreted to be α_{1A}- and α_{1B}-subtypes. In order to determine the pK_d values of [^{3}H]prazosin for these two subtypes, an experimental design using combined saturation and competition experiments was applied. The curves constructed were: a plain saturation curve for [^{3}H]prazosin, a saturation curve for [^{3}H]prazosin in the presence of 60 nM of 5-methyl-urapidil, and a saturation curve in the presence with 20 μM WB4101. The fourth simultaneously constructed curve was a competition curve of 5-methyl-urapidil at a fixed concentration of [^{3}H]prazosin (0.6 nM). The results are shown in Fig. 1A–B, and as can be seen in Table 1, the pK_d values of [^{3}H]prazosin were 9.2 ± 0.3 and 9.8 ± 0.2 for the α_{1A}- and α_{1B}-adrenoceptors, respectively. The pK_i-values of 5-methyl-urapidil determined from these experiments were 9.1 ± 0.14 and 7.2 ± 0.18 for the α_{1A}- and α_{1B}-adrenoceptors, respectively (mean ± S.E.M., $n = 3$). These latter values are almost identical to the pK_i values of 5-methyl-urapidil obtained from the competition experiments in the cerebral cortex (Section

Table 1
Drug pK_i values determined from competition experiments at α_{1A}-, α_{1B}- and adrenal gland α_1-adrenoceptors labelled by ~0.6 nM of [^{3}H]prazosin in membranes from pig cerebral cortex, liver, adrenal gland and aorta

Drug	Cerebral cortex (pK_i)		*n*	Liver (pK_i)	*n*	Adrenal gland (pK_i)	*n*	Aorta (pK_i)	*n*
	α_{1A}	α_{1B}		α_{1A}		$\alpha_{1\text{-adrenal}}$		α_{1B}	
methiothepin	10.2 ± 0.28	9.3 ± 0.23	3	9.3 ± 0.15	4	8.1 ± 0.39	3		
WB4101	10.1 ± 0.37	8.5 ± 0.29	18	9.3 ± 0.27	12	7.8 ± 0.19	3	8.7 ± 0.22	3
risperidone	9.3 ± 0.19	8.5 ± 0.13	3	8.9 ± 0.19	3	7.8 ± 0.13	3		
5-methyl-urapidil	9.1 ± 0.23	7.2 ± 0.29	4	9.0 ± 0.30	5	7.5 ± 0.17	2	6.9 ± 0.22	3
spiperone	8.3 ± 0.14	8.3 ± 0.05	3	7.9 ± 0.04	3	7.2 ± 0.45	4		
clozapine	8.2 ± 0.08	7.4 ± 0.17	3	8.5 ± 0.13	3	6.2 ± 0.15	3		
chlorpromazine	n.d.	n.d.	0	8.1 ± 0.25	4	6.8 ± 0.38	4		
thioridazine	7.8 ± 0.27	7.8 ± 0.17	4	7.9 ± 0.27	5	6.4 ± 0.26	5		
oxymetazoline	7.7 ± 0.31	6.8 ± 0.38	4	7.5 ± 0.07	3	n.d.	0		
S(+)-niguldipine	7.7 ± 0.06	6.5 ± 0.13	4	7.4 ± 0.24	5	5.6 ± 0.47	4		
SKF104856	7.3 ± 0.03	7.5 ± 0.14	3	7.2 ± 0.09	4	6.6 ± 0.33	4	7.6 ± 0.04	3
BMY7378	7.1 ± 0.11	6.8 ± 0.06	3	6.8 ± 0.05	4	6.2 ± 0.60	4	6.4 ± 0.14	3
(−)-noradrenaline	4.8 ± 0.11	5.6 ± 0.24	3	5.2 ± 0.15	4	see Table 2			
[^{3}H]prazosin	9.2 ± 0.30	9.8 ± 0.20	3	9.2 ± 0.28	4	9.6 ± 0.22	4		

3.4, Table 1). The B_{max} values were determined to be 60 ± 14 fmol/mg protein of α_{1A}-, and 56 ± 1 fmol/mg protein of α_{1B}-adrenoceptors, corresponding to 51 ± 6% α_{1A}- and 49 ± 6% α_{1B}-adrenoceptors (mean ± S.E.M., $n = 3$).

3.2. Determination of pK_d value of [^{3}H]prazosin for α_{1A}-adrenoceptors in the pig liver

In order to probe α_1-adrenoceptors in liver membranes, we first evaluated WB4101 and 5-methyl-urapidil in preliminary competition experiments. The competition experiments indicated a high affinity for both drugs with the competition curves being monophasic. This indicates that the pig liver contained only α_{1A}-adrenoceptors. To define the pK_d-value of [^{3}H]prazosin for these receptors, saturation experiments for [^{3}H]prazosin were performed. WB4101 at 20 μM was used to define the nonspecific binding (Fig. 2A). The pK_d-value of [^{3}H]prazosin for α_{1A}-adrenoceptors in the liver membranes was determined to be 9.2 ± 0.28 (Table 1). The B_{max} value was determined to be 68 ± 9 fmol/mg protein (mean ± S.E.M., $n = 4$).

3.3. Determination of the pK_d value of [^{3}H]prazosin for the putative α_1-adrenoceptor in the pig adrenal gland

For the receptor labelled by [^{3}H]prazosin in the adrenal gland, both WB4101 and 5-methyl-urapidil showed fairly low affinities (Table 1 and Fig. 3B). However, 20 μM of WB4101 was a high enough concentration for blocking the specific binding. In saturation experiments performed on the membranes of the adrenal gland the pK_d-value of [^{3}H]prazosin was determined to be 9.6 ± 0.22 (Table 1 and Fig. 3A). The B_{max} value was determined to be 4.2 ± 1.2 fmol/mg protein (mean ± S.E.M., $n = 4$).

3.4. Determination of pK_i values of substances competing for [^{3}H]prazosin binding in pig cerebral cortex membranes

In order to determine the pK_i values of 13 different substances for the α_1-adrenoceptor subtypes in the cerebral cortex, competition studies were performed using ~0.6 nM [^{3}H]prazosin as a labelled ligand. In each experiment, three competition curves were obtained simultaneously,

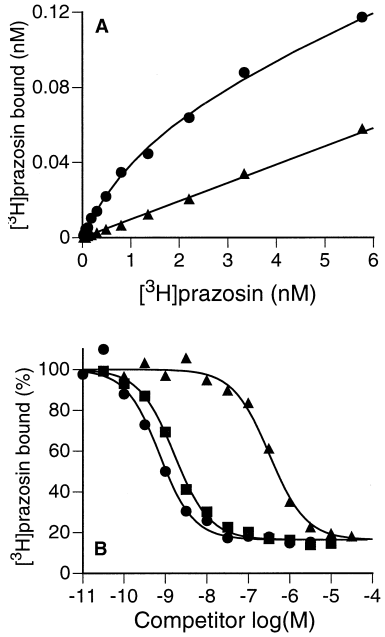

Fig. 2. Shown in (A) saturation experiment performed on pig liver membranes using [^{3}H]prazosin as labelled ligand. Total ligand binding (●) and the binding in the presence of 20 μM WB4101 (▲). Shown in (B) are competition curves of drugs competing at α_{1A}-adrenoceptors obtained by using 0.6 nM [^{3}H]prazosin and various concentrations of WB4101 (●), 5-methyl-urapidil (■) and BMY7378 (▲). The experiments of (A) and (B) are from different times.

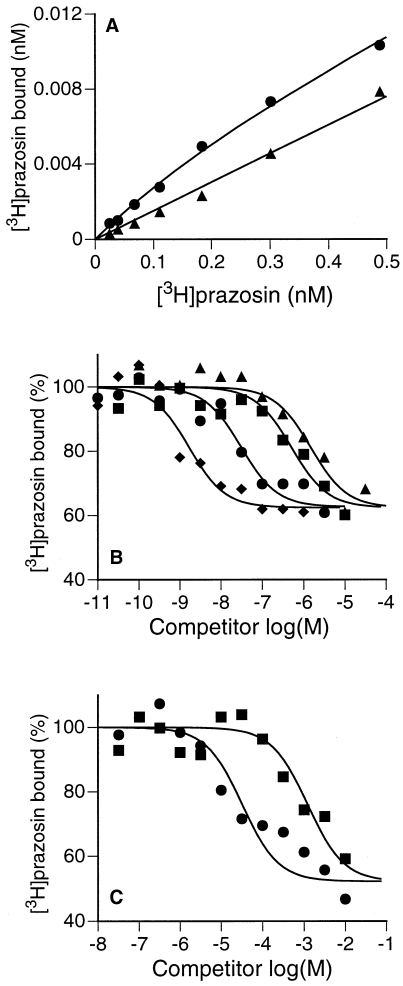

Fig. 3. Saturation and competition experiments performed on pig adrenal gland membranes. Shown in (A) are saturation curves using [^{3}H]prazosin as labelled ligand, total ligand binding (●) and the binding in the presence of 20 μM WB4101 (▲). Shown in (B) competition curves of prazosin (♦), WB4101 (●), 5-methyl-urapidil (■), and BMY7378 (▲) using a fixed concentration of [^{3}H]prazosin. Shown in (C) are competition curves of (−)-NA (●) and (+)-NA (■) using a fixed concentration of [^{3}H]prazosin. The experiments of (A)–(C) are from three different times.

one curve for the test compound, one for WB4101, and one for the test compound in the presence of 3 nM of WB4101. The competition curve in the presence of 3 nM of WB4101 represents mainly competition at α_{1B}-adrenoceptors while the non-blocked curve represents competition at both α_{1A}- and α_{1B}-adrenoceptors. Thus, the three competition curves in combination contain enough information to accurately determine the pK_i values of any drug tested for both α_{1A}- and α_{1B}-adrenoceptors. The results from the experiments performed in the pig cerebral cortex are shown in Fig. 1C and in Table 1. As can be seen in Fig. 1C, the competition curve for WB4101 is biphasic. The pK_i values were determined to be 10.1 ± 0.36 and 8.5 ± 0.28 for the α_{1A}- and α_{1B}-adrenoceptors, respectively (mean ± S.E.M., $n = 18$). The pK_i values of the test compounds are shown in Table 1. The most α_{1A}-selective drug found was 5-methyl-urapidil (70-fold α_{1A}-selective). In these experiments the proportions of sites were determined to be $64 \pm 14\%$ α_{1A}-adrenoceptors and $36 \pm 14\%$ α_{1B}-adrenoceptors, respectively (mean ± S.E.M., $n = 18$).

3.5. Determination of pK_i values of substances competing for [^{3}H]prazosin binding in pig liver

In order to identify the α_1-adrenoceptor subtype present in the pig liver [^{3}H]prazosin was used as a labelled ligand, and competition curves for 12 substances were obtained. The resulting pK_i values are shown in Table 1. As can be seen in Fig. 2, the competition curves of WB4101 and 5-methyl-urapidil were monophasic and showed high affinity while the competition curve of BMY7378 was monophasic and showed low affinity. This indicates that only the α_{1A}-subtype of α_1-adrenoceptors was present in the liver.

3.6. Determination of pK_i values of substances competing for [^{3}H]prazosin binding in pig adrenal gland

The same drugs tested in the cerebral cortex and liver were also tested in the adrenal gland. All the competitors showed fairly low affinities (Table 1). However, the order of potencies of the competing drugs were similar to their order of potencies at α_{1A}- and α_{1B}-adrenoceptors. In addition, unlabelled prazosin showed high affinity, the pK_i being 9.6, indicating that the labelled site is indeed an α_1-adrenoceptor. To further characterize the adrenal gland receptor, (−)-noradrenaline, (+)-noradrenaline, (−)-adrenaline and (+)-adrenaline were tested. If the labelled receptor was an adrenoceptor, then the affinity of the (−)-forms would be higher than for the (+)-forms, due to the stereospecificity of these compounds. As shown in Table 2, the pK_i value of (−)-noradrenaline was 5.3 and the pK_i value of (+)-noradrenaline was 3.8 (see also Fig. 3C). The pK_i value of (−)-adrenaline was 5.5 and the pK_i value of (+)-adrenaline was 4.6. This means 29 times higher affinity for the (−)-noradrenaline enantiomere, and about eight times higher affinity for the (−)-adrenaline enantiomere.

Table 2
Drug pK_i values determined from competition experiments on adrenal α_1-adrenoceptors labelled with ~0.6 nM of [^{3}H]prazosin in membranes from pig adrenal gland

Drug	Adrenal gland (pK_i) $\alpha_{1\text{-adrenal}}$	n
(−)-noradrenaline	5.3 ± 0.33	8
(+)-noradrenaline	3.8 ± 0.25	3
(−)-adrenaline	5.4 ± 0.59	4
(+)-adrenaline	4.6 ± 0.25	3
prazosin	9.6 ± 0.21	4

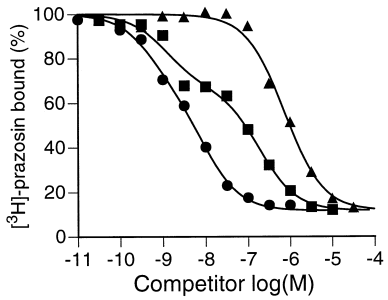

Fig. 4. Competition experiment for drugs competing for [^{3}H]prazosin binding to pig spleen membranes. Membranes were incubated with 0.6 nM [^{3}H]prazosin and various concentrations of WB4101 (●), 5-methyl-urapidil (■) and BMY7378 (▲).

3.7. Determination of pK_i values of substances competing for [^{3}H]prazosin binding in pig aorta

In the aorta the four subtype selective substances WB4101, 5-methyl-urapidil, SKF104856 and BMY7378 were tested. The competition curves were monophasic, and the pK_i-values corresponded best to the α_{1B}-adrenoceptor. Therefore, the affinity constant of [^{3}H]prazosin for the cerebral cortex α_{1B}-adrenoceptor was used for calculating the pK_i-values. The results are shown in Table 1.

3.8. Determination of pK_i values of substances competing for [^{3}H]prazosin binding in pig spleen.

The same drugs tested in the aorta were also tested in the spleen, in competition with 0.6 nM of [^{3}H]prazosin. The competition curves for WB4101 and 5-methyl-urapidil were biphasic and were resolved into two-site fits, while the competition curves for SKF104856 and BMY7378 were monophasic and were resolved into one-site fits (Fig. 4), showing low affinity (Table 3). It was concluded that the site showing high affinity for WB4101 and 5-methyl-urapidil (Table 3) represented the α_{1A}-subtype, since the drug pK_i values were similar to the cerebral cortex and liver α_{1A}-adrenoceptors (Table 1). The site showing low affinity for WB4101 and 5-methyl-urapidil seemed to correspond to the α_{1B}-subtype. The proportion of the sites were calculated to be $37 \pm 8\%$ α_{1A}-sites and $63 \pm 8\%$ α_{1B}-sites (mean $\pm$ S.E.M., $n = 5$). The affinity constants of [^{3}H]prazosin for the cerebral cortex α_{1A}- and α_{1B}-adrenoceptors were used to calculate the pK_i-values. Since in each experiment all four drugs were tested simultaneously, and drug pK_i values were calculated simultaneously, the proportions of the α_{1A}- and α_{1B}-adrenoceptors were defined by the two drugs that were fitted into a two-site model.

Table 3
Drug pK_i values determined from competition experiments on α_{1A}- and α_{1B}-adrenoceptors labelled by ~0.6 nM of [^{3}H]prazosin in membranes from pig spleen and heart

Drug	α_{1A} (pK_i)	Shared (pK_i)	α_{1B} (pK_i)	*n*
(A) Spleen				
WB4101	9.6 ± 0.62		8.8 ± 0.18	5
5-methyl-urapidil	9.4 ± 0.29		7.4 ± 0.51	5
SKF104856		7.6 ± 0.09		3
BMY7378		6.8 ± 0.09		4
(B) Heart				
WB4101	10.1 ± 0.28		8.6 ± 0.34	3
5-methyl-urapidil	8.9 ± 0.58		6.9 ± 0.32	3
SKF104856		7.1 ± 0.01		3
BMY7378		6.2 ± 0.14		3

3.9. Determination of pK_i values of substances competing for [^{3}H]prazosin binding in pig heart

In the heart, we tested the same drugs as in the aorta and spleen. The competition curves for WB4101 and 5-methyl-urapidil were clearly biphasic, with a similar pattern as in the cerebral cortex and the spleen. The competition curves of SKF104856 and BMY7378 were monophasic and of low affinity (Table 3). Altogether, this indicates that both α_{1A}- and α_{1B}-adrenoceptors, but not the α_{1D}-subtype, were present in the heart. The proportions of the sites were calculated to be $53 \pm 6\%$ α_{1A}- and $47 \pm 6\%$ α_{1B}-adrenoceptors (mean $\pm$ S.E.M., $n = 3$). The pK_i values were calculated as described in the section above.

4. Discussion

In the present paper, we report the first broad pharmacological characterization of the pig α_1-adrenoceptor subtypes. Since none of the porcine α_1-adrenoceptors have been cloned, we studied the α_1-adrenoceptors labelled by [^{3}H]prazosin in different pig tissues. The results indicate that the pig α_{1A}- and α_{1B}-subtypes are pharmacologically similar to those in e.g., human and rat. In addition, the pig seems to express an α_1-adrenoceptor subtype in the adrenal gland which shows pharmacological characteristics that distinguish if from the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors previously described pharmacologically in other species.

The pharmacological characterization of the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes has been fairly difficult (Hieble et al., 1995) and is still continuing (Ford et al., 1997). Here, only a few important pharmacological characteristics for the three subtypes will be mentioned. From investigations in several species the drugs WB4101, 5-methyl-urapidil and niguldipine have been shown to be α_{1A}- selective over the α_{1B}- and α_{1D}-subtypes (Michel et al., 1995). The α_{1B}-subtype has historically been defined by its low affinity for various drugs and its sensitivity towards alkylation by chloroethylclonidine. It has been proposed that the drugs, spiperone and risperidone, are α_{1B}- to α_{1A}-selective in the rat (Michel et al., 1989; Sleight et al., 1993; Bylund et al., 1994). There are a few

drugs that have been proposed to be selective for the α_{1D}-adrenoceptor subtype. BMY7378 was shown to be α_{1D}-selective among the human recombinant α_1-adrenoceptors (Saussy et al., 1996), and it showed high affinity for the α_{1D}-adrenoceptor present in the rat aorta (Testa et al., 1995; Deng et al., 1996). In addition, SKF104856 has been shown to be α_{1D}-selective (Testa et al., 1995; Hieble et al., 1995).

In the pig cerebral cortex two populations of α_1-adrenoceptors were detected. Due to the presence of both high and low binding affinities for 5-methyl-urapidil and WB4101, and the low affinity of BMY7378, we concluded that these sites corresponded to the α_{1A}- and α_{1B}-adrenoceptor subtypes (Table 1). The proportions of the sites in the cerebral cortex were 64% α_{1A}- and 36% α_{1B}-adrenoceptors, respectively. It can be noted that in the present study spiperone and risperidone, which are α_{1B}- to α_{1A}-selective in the rat (Michel et al., 1989; Sleight et al., 1993; Bylund et al., 1994), was not found to be α_{1B}-selective in the pig.

In the pig liver we detected only one α_1-adrenoceptor, which was identified as the α_{1A}-subtype by its high affinity for WB4101 and 5-methyl-urapidil (Fig. 2 and Table 1). The low affinity of BMY7378 in this tissue support the conclusion that the α_{1D}-adrenoceptor was not present in the pig liver. Previously, it has been observed that there is a considerable species variation in what subtype of α_1-adrenoceptors that is expressed in the liver. Thus, rat (Han and Minneman, 1991; Garcia-Saintz et al., 1992), mouse and hamster (Garcia-Saintz et al., 1994) express the α_{1B}-subtype, whereas rabbit (Garcia-Saintz et al., 1992; Taddei et al., 1993), guinea pig, dog and human express predominantly the α_{1A}-subtype (see Garcia-Saintz et al., 1995). Surprisingly, WB4101 showed lower affinity for the α_{1A}-site in the liver (pK_i 9.3) than for the α_{1A}-site in the cerebral cortex (pK_i 10.1). However, speaking in favour of that both these sites represented α_{1A}-adrenoceptors was the fact that 5-methyl-urapidil, which is a compound with high affinity only for the α_{1A}-subtype, showed high affinity both in the cerebral cortex and the liver (pK_i 9.1 and 9.0, respectively). In addition, the correlation analysis for 12 drugs showed a very high correlation coefficient ($r^2 = 0.95$) between these two sites (Fig. 5A). Different affinities of WB4101 for different α_{1A}-populations have been previously reported (Ford et al., 1997), but at present the significance of these observed differences is not clear.

In the spleen and heart, both α_{1A}- and α_{1B}-adrenoceptors were present, while in the aorta only the α_{1B}-subtype was present. The identification of the subtypes was based on that WB4101 and 5-methyl-urapidil showed high affinity for the α_{1A}-sites, and low affinity for the α_{1B}-sites. The potential presence of the α_{1D}-subtype was excluded, since BMY7378 and SKF10485, which are supposed to be α_{1D}-selective (Ruffolo et al., 1995), only showed single low affinities in all three tissues (Tables 1 and 3). Also, clearly indicating that the α_{1B}-sites was different from the adrenal

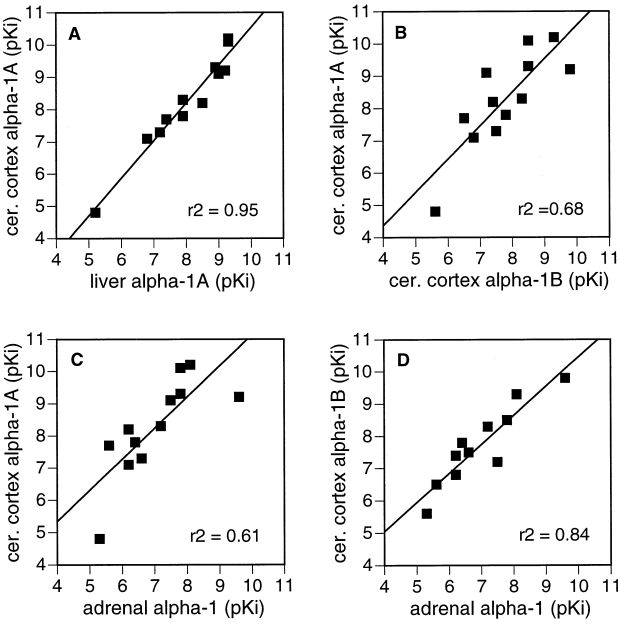

Fig. 5. Correlations of pK_i values for 12 drugs, taken from Table 1, for pig α_1-adrenoceptor populations in cerebral cortex, liver and adrenal gland. Inserted in the figures are the correlation coefficients r^2.

gland α_1-adrenoceptor, the affinity of WB4101 was higher for the α_{1B}-sites in the spleen, heart and aorta compared to its affinity for the adrenal gland α_1-adrenoceptor.

Pilot experiments in the adrenal gland, using about 0.6 nM [^{3}H]prazosin, indicated that an α_1-adrenoceptor was present in this tissue. However, the pK_i values of the drugs tested were different from their pK_i values for the α_{1A}-and α_{1B}-adrenoceptors (Table 1). In the correlation analysis for the 12 drugs the adrenal gland α_1-adrenoceptor was clearly different from the cerebral cortex α_{1A}-subtype ($r^2 = 0.61$), and moderately different from the α_{1B}-subtype ($r^2 = 0.84$) (Fig. 5C–D). The best drug used to discriminate between the adrenal gland α_1-adrenoceptor and the α_{1B}-adrenoceptor was thioridazine, which had 25-fold lower affinity for the adrenal gland receptor than for any of the cerebral cortex α_{1A}- or α_{1B}-adrenoceptors (Table 1). It can be noted that WB4101 had a 200-fold lower affinity for the adrenal gland α_1-adrenoceptor than for the α_{1A}-adrenoceptor from cerebral cortex. The drug with the highest affinity in the adrenal gland was prazosin (pK_i 9.6), followed by methiothepin (pK_i 8.1). 5-methyl-urapidil had about the same affinity for the adrenal gland receptor (pK_i 7.5) as for the cerebral cortex α_{1B}-adrenoceptor (pK_i 7.2). All other drugs tested in the adrenal gland, including BMY7378 and SKF104856, showed moderately low affinity for this receptor (Table 1).

It can be noted that in the present study we did not detect any α_1-adrenoceptor population with high affinity for either BMY7378 or SKF104856. This indicates either that the α_{1D}-adrenoceptor was not detectable in the studied tissues, or that the pig adrenal gland α_1-adrenoceptor genetically corresponds to the α_{1d}-subtype. In the latter

case, the pharmacology of the pig α_{1D}-subtype is different from that of the α_{1D}-subtype in other species. For example, the pK_i value of BMY7378 for the pig adrenal gland α_1-adrenoceptor was determined to be 6.2. In contrast, the pK_i value of BMY7378 for the human α_{1D}-subtype was reported to be in the range 8.2 to 9.4 (Kenny et al., 1995; Saussy et al., 1996). Altogether, if the pig adrenal gland α_1-adrenoceptor represents the porcine ortholog of the α_{1D}-adrenoceptor, then this ortholog is a species variant showing low affinity for BMY7378 and SKF104856.

In order to verify that the high affinity binding site for [^{3}H]prazosin in the adrenal gland membranes was an α_1-adrenoceptor, we tested the cathecholamines (−)-epinephrine, (+)-epinephrine, (−)-norepinephrine and (+)-norepinephrine. If the receptor present in the adrenal gland was an adrenergic receptor, then the (−)-forms would stereoselectively show higher affinity than the (+)-forms for the receptor. The competition curves for (−)- and (+)-norepinephrine are shown in Fig. 3C, and in Table 2 are shown all the pK_i values for the (−)- and (+)-enantiomers of epinephrine and norepinephrine for the receptor labelled by [^{3}H]prazosin in the adrenal gland. It can be noted that 100 μM of Gpp(NH)p was present in the binding buffers. Gpp(NH)p uncouples G-protein coupled receptors, e.g., α_1-adrenoceptors, from their G-proteins, which may explain the overall fairly low affinities of the tested catecholamines (see the work of Uhlén et al., 1992). As can be seen in Table 2, the affinity of (−)-norepinephrine was 26 times higher than that of (+)-norepinephrine, and the affinity of (−)-epinephrine was eight times higher than that of (+)-epinephrine. These results speak in favour of the conclusion that the binding site for [^{3}H]prazosin in the adrenal gland membranes is indeed an α_1-adrenoceptor.

It has been postulated by other authors that there exists a fourth subtype of α_1-adrenoceptors. Muramatsu et al. (1990) proposed that a functional site in vascular smooth muscle, for which prazosin showed low affinity, represented an additional α_1-adrenoceptor and denoted it α_{1L}. Ohmura et al. (1992) suggested on the basis of pharmacological studies that a population of α_{1L} was present in rat vas deferens. Since the site detected by us in the pig adrenal gland shows high affinity for prazosin, this receptor does not seem to be the same as the α_{1L} receptor mentioned above.

In summary, in the present study we show that [^{3}H]prazosin labels both α_{1A}- and α_{1B}-adrenoceptors in the pig cerebral cortex, spleen and heart. In the liver it labelled only the α_{1A}-subtype, while in the aorta only the α_{1B}-subtype. Substances showing high selectivity for the pig α_{1A}- over the α_{1B}-subtype were 5-methyl-urapidil and WB4101. In the adrenal gland [^{3}H]prazosin labeled an α_1-adrenoceptor subtype with a pharmacological profile that has not previously been described. The adrenal gland α_1-adrenoceptor showed high affinity for prazosin, but relatively low affinities for WB4101, spiperone and BMY7378, indicating that it differed from the previously described α_{1A}-, α_{1B}- and α_{1D}-subtypes in other species. The best drug for discriminating between the cerebral cortex α_{1A}- and α_{1B}-subtypes and the adrenal gland α_1-adrenoceptor was thioridazine, which had 25-fold lower affinity for the latter receptor. The pig adrenal gland α_1-adrenoceptor may represent the porcine ortholog of the α_{1D}-adrenoceptor.

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References

Bylund, D.B., Eikenberg, D.C., Hieble, J.P., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Molinoff, P.B., Ruffolo, R.R., Trendelenburg, U., 1994. International union of pharmacology nomenclature of adrenoceptors. Pharmacol. Rev. 46, 121–136.

Deng, F.D., Chemtob, S., Varma, D.R., 1996. Characterization of α_1-adrenoceptor subtype in rat myocardium, aorta and other tissues. Br. J. Pharmacol. 119, 269–276.

Ford, A.P.D.W., Williams, T.J., Blue, D.R., Clarke, D.E., 1994. α_1-Adrenoceptor classification: sharpening Occam's razor. Trends Pharmacol. Sci. 15, 167–170.

Ford, A.P.D.W., Daniels, D.V., Chang, D.J., Gever, J.R., Jasper, J.R., Lesnick, J.D., Clarke, D.E., 1997. Pharmacological pleiotropism of the human recombinant α_{1A}-adrenoceptor: implications for α_1-adrenoceptor classification. Br. J. Pharmacol. 121, 1127–1135.

Garcia-Saintz, J.A., Romero-Avila, M.T., Hernandez, R.A., Marcia-Silva, M., Olivares-Reyes, A., Gonzales-Espinosa, C., 1992. Species heterogeneity of hepatic α_1-adrenoceptors: α_{1A}-, α_{1B}- and α_{1C}-subtypes. Biochem. Biophys. Res. Commun. 186, 760–767.

Garcia-Saintz, J.A., Casas-Gonzales, P., Romero-Avila, M.T., Gonzales-Espinosa, C., 1994. Characterization of the hepatic α_{1B}-adrenoceptors of rats, mice and hamsters. Life Sci. 54, 1995–2003.

Garcia-Saintz, J.A., Romero-Avila, M.T., Torres-Marquez, M.E., 1995. Characterization of the human liver α_1-adrenoceptors: predominance of the α_{1A}-subtype. Eur. J. Pharmacol. 289, 81–86.

Goetz, A.S., Lutz, M.W., Rimele, T.J., Saussy, D.L., 1994. Characterization of alpha-1 adrenoceptor subtypes in human and canine prostate membranes. J. Pharmacol. Exp. Ther. 271, 1228–1233.

Guarino, R.D., Perez, D.M., Piascik, M.T., 1996. Recent advances in the molecular pharmacology of the α_1-adrenergic receptors. Cell. Signal. 8, 323–333.

Han, C., Minneman, K.P., 1991. Interaction of subtype-selective antagonists with α_1-adrenergic receptor binding sites in rat tissues. Mol. Pharmacol. 40, 531–538.

Hieble, J.P., Bylund, D.B., Clarce, D.E., Eikenburg, D.C., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Ruffolo, R.R., 1995. International union of pharmacology: X. Recommendation for nomenclature of α_1-adrenoceptors: consensus update. Pharmacol. Rev. 47, 267–270.

Kenny, B.A., Chalmers, D.H., Philpott, P.C., Naylor, A.M., 1995. Characterization of an α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br. J. Pharmacol. 115, 981–986.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Michel, A.D., Loury, D.N., Whiting, R.L., 1989. Identification of a single α_1-adrenoceptor corresponding to the α_{1A}-subtype in rat submaxillary gland. Br. J. Pharmacol. 98, 883–889.

Michel, M.C., Kenny, B., Schwinn, D.A., 1995. Classification of α_1-adrenoceptor subtypes. Naunyn Schmiedenberg's Arch. Pharmacol. 352, 1–10.

Muramatsu, I., Ohmura, T., Kigoshi, S., Hashimoto, S., Oshita, M., 1990. Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. Br. J. Pharmacol. 99, 197–201.

Ohmura, T., Oshita, M., Kigoshi, S., Muramatsu, I., 1992. Identification of α_1-adrenoceptor subtypes in the rat vas deferens: binding and functional studies. Br. J. Pharmacol. 107, 697–704.

Oshita, M., Kigoshi, S., Muramatsu, I., 1993. Pharmacological characterization of two distinct α_1-adrenoceptor subtypes in rabbit thorasic aorta. Br. J. Pharmacol. 108, 1071–1076.

Piascik, M.T., Smith, M.S., Soltis, E.E., Perez, D.M., 1994. Identification of the mRNA for the novel α_{1D}-adrenoceptor and two other α_1-adrenoceptors in vascular smooth muscle. Mol. Pharmacol. 46, 30–40.

Price, D.T., Lefkowitz, R.J., Caron, M.G., Berkowitz, D., Schwinn, D.A., 1993. Localization of mRNA for three distinct α_1-adrenoceptor subtypes in human tissues. Implication for human α-adrenergic physiology. Mol. Pharmacol. 45, 171–175.

Ruffolo, R.R., Bondinell, W., Hieble, J.P., 1995. α- and β-adrenoceptors: from gene to clinic: II. Structure activity relationships and therapeutic implications. J. Med. Chem. 38, 3681–3716.

Saussy, D.L., Goetz, A.S., Queen, K.L., King, H.K., Lutz, M.W., Rimele, T.J., 1996. Structure activity relationships of a series of buspirone analogs at alpha-1 adrenoceptors: further evidence that rat aorta alpha-1 adrenoceptors are of the alpha-1D-subtype. J. Pharmacol. Exp. Ther. 278, 136–144.

Scofield, M.A., Liu, F., Abel, P., Jeffries, W.B., 1995. Quantification of steady state expression of mRNA for alpha-1 adrenergic receptor subtypes using reverse transcription and competitive polymerase chain reaction. J. Pharmacol. Exp. Ther. 275, 1035–1042.

Sleight, A.J., Koek, W., Bigg, C.H., 1993. Binding of antipsychotic drugs at α_{1A}- and α_{1B}-adrenoceptors: risperidone is selective for the α_{1B}-adrenoceptors. Eur. J. Pharmacol. 238, 407–410.

Taddei, C., Poggesi, E., Leonardi, A., Testa, R., 1993. Affinity of different α_1-agonists and antagonists for α_1-adrenoceptors of rabbit and rat liver membranes. Life Sci. 57, PL159–PL163.

Testa, R., Destefani, C., Guarneri, L., Poggesi, E., Simonazzi, I., Taddei, C., Leonardi, A., 1995. The α_{1D}-adrenoceptor subtype is involved in the noradrenaline induced contractions of rat aorta. Life Sci. 53, PL177–PL181.

Uhlén, S., Wikberg, J.E.S., 1991a. Rat spinal cord α_2-adrenoceptors are of the α_{2A}-subtype: comparison with α_{2A}- and α_{2B}-adrenoceptors in rat spleen, cerebral cortex and kidney using ^{3}H-RX821002 ligand binding. Pharmacol. Toxicol. 69, 341–350.

Uhlén, S., Wikberg, J.E.S., 1991b. Delineation of rat kidney α_{2A} and α_{2B} adrenoceptors with ^{3}H-RX821002 radioligand binding: computer modelling reveals that guanfacine is an α_{2A}-selective compound. Eur. J. Pharmacol. 202, 235–243.

Uhlén, S., Xia, Y., Chhajlani, V., Felder, C.C., Wikberg, J.E.S., 1992. [^{3}H]MK912 binding delineates two α_2-adrenoceptor subtypes in rat CNS one of which is identical with the cloned pA2d α_2-adrenoceptor. Br. J. Pharmacol. 106, 986–995.