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Characterization of the α_{1A}-Adrenoceptors of Guinea Pig Liver Membranes: Studies Using 5-[^{3}H]Methylurapidil

J. ADOLFO GARCÍA-SÁINZ and M. TERESA ROMERO-AVILA

Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, 04510 México City, México

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SUMMARY

Binding of 5-[^{3}H]methylurapidil to guinea pig liver membranes was rapid, saturable, and reversible. Scatchard analysis of saturation isotherms indicated a single class of binding sites with a K_d of 0.86 nM and a B_{max} of 36 fmol/mg of protein. Preincubation of the membranes with chlorethylclonidine did not alter significantly the binding parameters for 5-[^{3}H]methylurapidil. Binding competition experiments were performed, and the order of potency for agonists was oxymetazoline > epinephrine > norepinephrine ≫ methoxamine; for antagonists, the potency order was (+)-niguldipine ≥ 5-methylurapidil = prazosin = WB4101 > benoxathian ≥ phentolamine ≥ (−)-niguldipine. The binding affinity for epinephrine was modulated by the hydrolysis-resistant GTP analogue guanosine-5′-(β,γ-imido)triphosphate. The pharmacological profile of the 5-[^{3}H]methylurapidil binding sites of guinea pig liver differs markedly from those of the cloned α_1-adrenoceptors (i.e., α_{1B}-, α_{1C}-, and $\alpha_{1A/D}$-adrenoceptors) and resembles that of the classical α_{1A} receptor subtype.

It is now clear that α_1-adrenoceptors constitute an heterogeneous family of receptors. The existence of two subtypes of α_1-adrenoceptors, the α_{1A} and α_{1B} subtypes, was initially suggested by pharmacological criteria (1, 2). The α_{1B}-adrenoceptor has now been cloned and expressed (3). However, the cloning of the α_{1A}-adrenoceptor has been elusive. Nevertheless, during attempts to isolate the α_{1A}-adrenoceptor gene two other receptors of this family were cloned, i.e., the α_{1C}- and the $\alpha_{1A/D}$-adrenoceptors (4–7). The α_{1C}-adrenoceptor has a pharmacological profile and a tissue distribution that differ markedly from those expected for α_{1A}-adrenoceptors (4, 8). Later, another receptor was cloned by Lomasney *et al.* (5); it showed pharmacological similarities to and the tissue distribution expected for the α_{1A} receptor (it was named the α_{1A}-adrenoceptor). However, another receptor, essentially identical to that described earlier (5) (except for two codons), was cloned; it was named the α_{1D}-adrenoceptor because it showed some peculiar pharmacological features. Additional studies by Schwinn and Lomasney (7) indicated that the cloned α_{1A}-adrenoceptor showed an atypically low affinity for some selective compounds [(+)-niguldipine, 5-methylurapidil, and benoxathian]; the compromise designation $\alpha_{1A/D}$ was suggested (7). In summary, at least four members constitute this family of receptors, i.e., the cloned α_{1B}-, α_{1C}-, and $\alpha_{1A/D}$-adrenoceptors and the "classical" α_{1A} receptor.

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Interestingly, we recently observed that there is considerable variation in the subtypes of α_1-adrenoceptor expressed in liver cells of different species (9). Thus, rat hepatocytes express α_{1B}- (9–11), rabbit hepatocytes α_{1C}- (8, 9), and guinea pig hepatocytes α_{1A}-adrenoceptors (9, 12). Here we present the characterization of the α_1-adrenoceptors present in guinea pig liver membranes using 5-[^{3}H]methylurapidil; our data indicate that these receptors have pharmacological characteristics that are similar to those of classical α_{1A}-adrenoceptors and differ markedly from those of the $\alpha_{1A/D}$ receptor.

Materials and Methods

(−)-Epinephrine, (−)-norepinephrine, oxymetazoline, and prazosin were obtained from Sigma Chemical Co. Benoxathian and WB4101 were from RBI. The following compounds were generous gifts from the companies indicated: 5-[^{3}H]methylurapidil (40 Ci/mmol), 5-methylurapidil, (+)-niguldipine, and (−)-niguldipine (Byk Gulden); phentolamine (Ciba-Geigy); and methoxamine (Burroughs Wellcome).

Liver membranes, from male guinea pigs (225–300 g), were obtained by the method of Neville (13) up to step 11. Membranes were washed and resuspended in the buffer used for the binding studies (50 mM Tris, 10 mM $MgCl_2$, pH 7.5). Binding studies were performed by incubating the membranes (500 μg) with the radioactive ligand, alone or with the indicated agents, in a total volume of 0.5 ml for 20 min (unless otherwise indicated), in a water bath shaker at 25°. At the end of the incubation, 10 ml of ice-cold buffer were added to the membrane suspension, and the membranes were immediately filtered on GF/C filters (Whatman) and washed three times (10 ml each time) with the same buffer. Saturation experiments were performed using 0.25–12 nM 5-[^{3}H]methylurapidil, and kinetic and binding competition studies used

ABBREVIATION: Gpp(NH)p, guanosine-5′-(β,γ-imido)triphosphate.

3–4 nM concentrations of the radioactive ligand (70–75% receptor occupation). Nonspecific binding was evaluated in the presence of 1 μM prazosin; specific binding represented 60–70% at the K_d value. Binding saturation and competition data were analyzed using the KINETIC, EBDA, and LIGAND (14) programs (Biosoft-Elsevier). Two-state fits were utilized only when this more complex model significantly improved the goodness of fit. Hill coefficients or slope factors were calculated as described (15, 16). K_i values were calculated according to the method of Cheng and Prusoff (17). Protein was quantified by the method of Lowry *et al.* (18), using bovine serum albumin as the standard.

Results

Binding of 5-[^{3}H]methylurapidil was rapid, saturable, and reversible (Figs. 1 and 2). Binding kinetic studies indicated a k_1 of $1.49 \pm 0.09 \times 10^8$ M^{-1} min^{-1} and a k_{-1} of 0.11 ± 0.02 min^{-1}, with a resulting K_d of 0.75 ± 0.08 nM (means ± standard errors, four experiments). Scatchard analysis of saturation isotherm data resulted in straight lines, indicating a single class of binding sites with a K_d for 5-[^{3}H]methylurapidil of 0.86 ± 0.06 nM and a receptor density of 36 ± 4 fmol/mg of protein (results are means ± standard errors of 12 experiments using different membrane preparations). Representative data are presented in Fig. 2.

The pharmacological properties of these binding sites were next examined. Chlorethylclonidine is an irreversible antagonist that inactivates α_{1B}-, α_{1C}-, and $\alpha_{1A/D}$-adrenoceptors but not receptors of the α_{1A} subtype (3, 5–8, 10). To test the sensitivity of guinea pig liver receptors to this alkylating agent, membranes were incubated in the absence or presence of 100 μM chlorethylclonidine for 15 min at 37°. After this incubation, the membranes were washed and saturation experiments were performed. A representative experiment is shown in Fig. 3. As can be observed, incubation with chlorethylclonidine induced almost no change in the affinity of these receptors for 5-[^{3}H] methylurapidil or in the number of sites detected with this ligand. The data from the experiments were as follows: K_d values of 0.75 ± 0.06 and 0.84 ± 0.15 nM and B_{max} values of 25 ± 2 and 26 ± 3 fmol/mg of protein for membranes incubated in the absence and presence of chlorethylclonidine, respectively (means ± standard errors, four experiments).

Binding competition experiments with agonists and antagonists were next performed. Representative displacement experiments are presented in Figs. 4 (agonists) and 5 (antagonists), and the data are presented in Table 1. It can be seen that the order of potency for agonists was oxymetazoline > epinephrine > norepinephrine ≫ methoxamine. For antagonists, the potency order was (+)-niguldipine ≥ 5-methylurapidil = prazosin = WB4101 > benoxathian ≥ phentolamine ≥ (−)-niguldipine. The slopes of the competition curves obtained with agonists varied between 0.5 and 0.7, whereas those for antagonists were closer to 1 (0.75–0.90), suggesting that the sites labeled with 5-[^{3}H]methylurapidil have heterogeneous affinity for agonists but are essentially homogeneous in affinity for antagonists. Current ideas indicate that most G protein-coupled receptors exhibit two interconvertible states of affinity for agonists, i.e., high and low affinity, and that conversion is mediated via interaction with regulatory G proteins (19, 20). To test this concept, more detailed displacement studies were performed using epinephrine in the absence or presence of the hydrolysis-resistant GTP analogue Gpp(NH)p. Representative data are presented in Fig. 6, and the analysis of the data is presented in Table 2. It can be seen that the epinephrine displacement curve was rather shallow but became steeper in the presence of the GTP analogue (Fig. 6A). This was also clearly evident in the Hill analysis of the data (Fig. 6B; Table 2). LIGAND analysis of the data indicated that in the absence of guanine nucleotide the competition curve with epinephrine was best fitted to a two-site model, whereas in the presence of Gpp(NH)p a one-site fit was preferred. The K_d of the low affinity site in the absence of guanine nucleotide was similar to the K_d observed in the presence of Gpp(NH)p (Table 2), suggesting the conversion of the high affinity site to the low affinity site for epinephrine in the presence of the guanine nucleotide.

Discussion

5-[^{3}H]Methylurapidil has been previously used as a radioligand in binding studies with rat brain membranes and tissue sections (21, 22). This derivative of urapidil binds to α_{1A}-adrenoceptors and 5-hydroxytryptamine type 1A receptors in these preparations (21, 22) but not to purified rat liver mem-

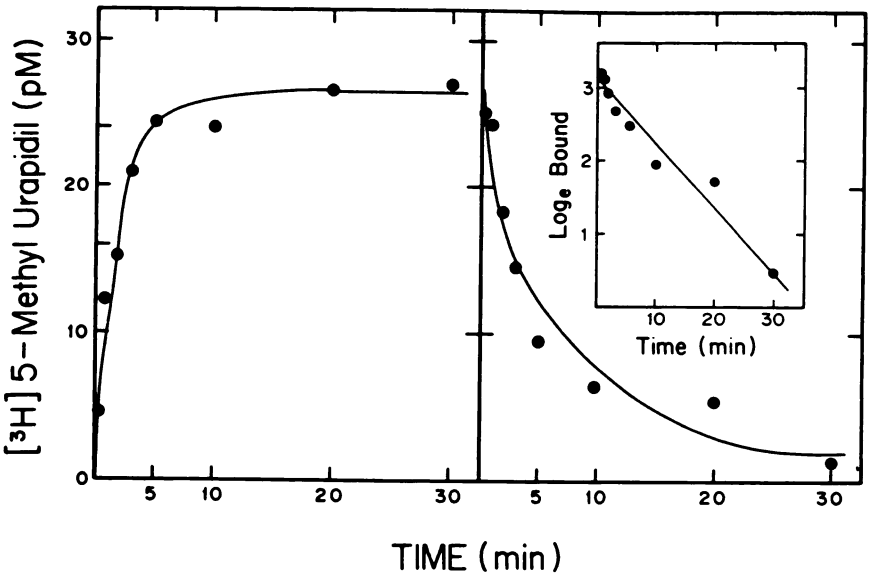

Fig. 1. Kinetics of 5-[^{3}H]methylurapidil binding to guinea pig liver membranes. *Left*, membranes (550 μg of protein) were prewarmed for 5 min in the absence or presence of 1 μM prazosin; 5-[^{3}H]methylurapidil (≈3 nM) was added and the reaction was stopped by filtration at the times indicated. *Right*, for reversal of binding, membranes were incubated as indicated above for 30 min, 1 μM prazosin was added to the tubes that did not contain this antagonist, and at the times indicated the reaction was stopped (*inset*, linear plot; $r = 0.97$). Plotted is a representative experiment that was replicated four times using different membrane preparations.

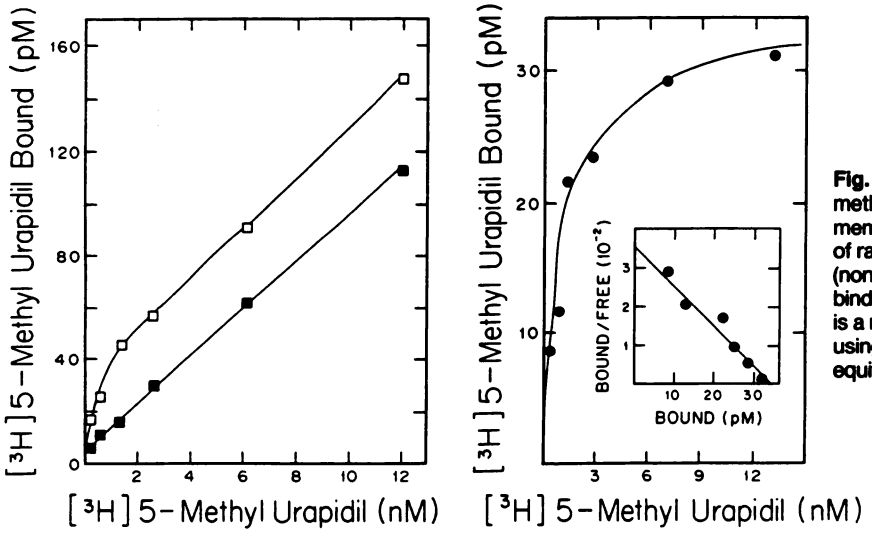

Fig. 2. Binding isotherms and Scatchard analysis of 5-[^{3}H] methylurapidil binding to guinea pig liver membranes. *Left*, membranes were incubated with increasing concentrations of radioligand in the absence (total binding) (□) or presence (nonspecific binding) (■) of 1 μM prazosin. *Right*, specific binding (●) and Scatchard plot (*inset*) are shown. Plotted is a representative experiment that was replicated 12 times using different membrane preparations; 1000 cpm are equivalent to 39.7 fmol of 5-[^{3}H]methylurapidil.

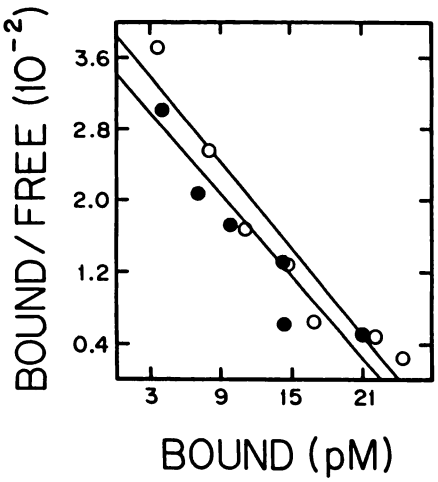

Fig. 3. Absence of effect of preincubation with chlorethylclonidine on 5-[^{3}H]methylurapidil binding to guinea pig liver membranes. Aliquots of membranes were divided into two groups and incubated as described in Materials and Methods, in the absence (○) or presence (●) of chlorethylclonidine. After this preincubation the membranes were washed by centrifugation/resuspension and 5-[^{3}H]methylurapidil binding was assayed. Plotted is the Scatchard transformation of saturation isotherms from a representative experiment that was replicated four times using different membrane preparations.

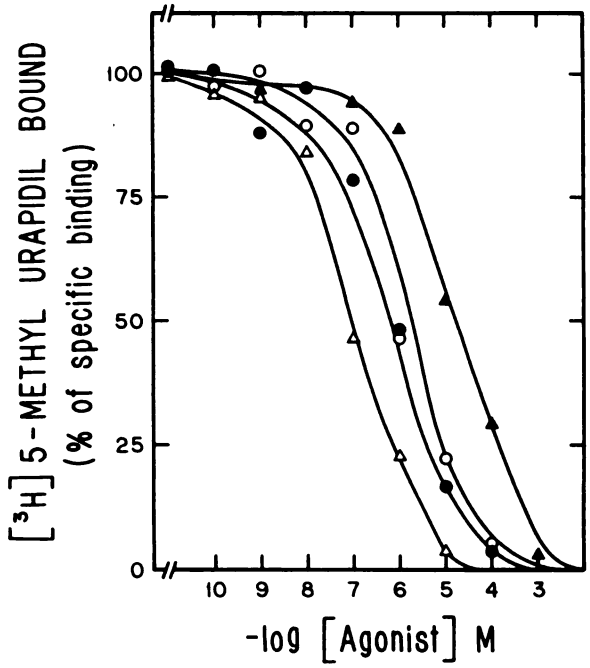

Fig. 4. Competition by agonists for 5-[^{3}H]methylurapidil binding sites. Membranes were incubated with ≈3 nM 5-[^{3}H]methylurapidil and varying concentrations of oxymetazoline (△), epinephrine (●), norepinephrine (○), or methoxamine (▲). Plotted is a representative experiment (total binding, 893 cpm; nonspecific binding, 402 cpm; specific binding, 491 cpm) that was replicated four times using different membrane preparations.

branes (α_{1B}-adrenoceptors) (22). Prazosin and (+)-niguldipine are competitive inhibitors of 5-[^{3}H]methylurapidil binding to α_{1A}-adrenoceptors (21, 22).

The present data are consistent with our previous observations suggesting that the α_1-adrenoceptors of guinea pig hepatocytes belong to the α_{1A} subtype (9, 12). 5-[^{3}H]Methylurapidil seems to be a suitable ligand for characterizing these receptors. This ligand showed high affinity and specificity and a relatively low level of nonspecific binding. The K_d values observed in kinetic (0.75 nM) and steady state, i.e., saturation (0.86 nM) (Fig. 1) and competition (1.2 nM) (Fig. 4; Table 1), studies were similar and in reasonable agreement with the K_i obtained in the phosphorylase studies in whole cells (3 nM) (9, 12) and in the binding competition studies using [^{3}H]bunazosin (0.75 nM) (9). The present data confirm that these receptors have very high affinity for this antagonist, which is in marked contrast to what has been observed for the cloned $\alpha_{1A/D}$-adrenoceptor (15–330 nM) (6, 7). The affinity observed for 5-methylurapidil in guinea pig liver is in agreement with that observed for α_{1A}-adrenoceptors in rat vas deferens and hippocampus (23). It should be mentioned that the number of sites detected with 5-[^{3}H]methylurapidil was lower than that obtained using [^{3}H] bunazosin, although the variation between membrane preparations was large. The reason for this difference is currently unknown but it does not seem to be due to the labeling of different receptors, because the K_i values for 5-methylurapidil, prazosin, and bunazosin were essentially identical using these radioactive ligands (see Refs. 9 and 12).

Pretreatment with chlorethylclonidine did not block the α_1-

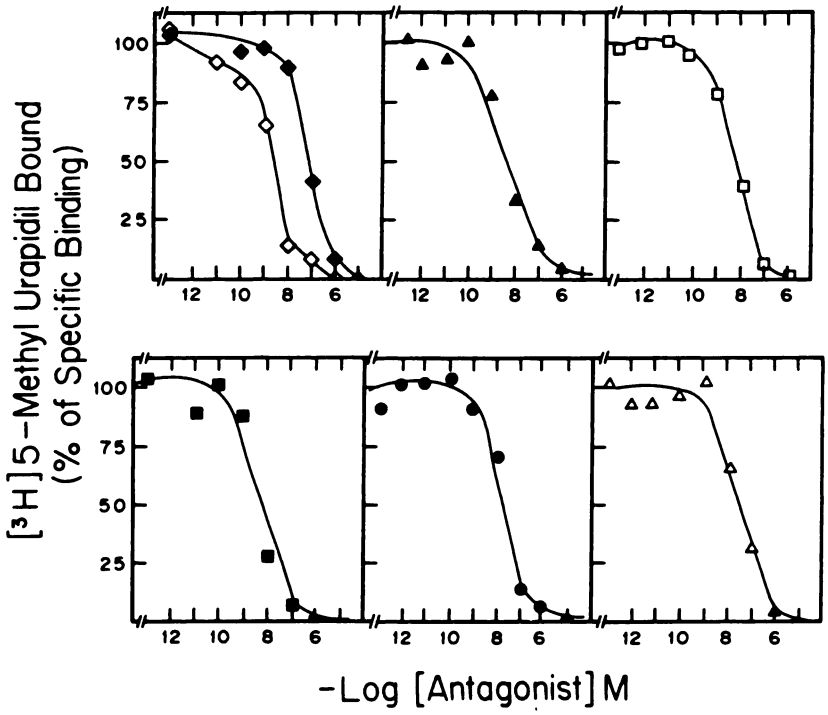

Fig. 5. Competition by antagonists for 5-[^{3}H]methylurapidil binding sites. Membranes were incubated with ≈3 nM 5-[^{3}H]methylurapidil and varying concentrations of (+)-niguldipine (◇), (−)-niguldipine (◆), 5-methylurapidil (▲), prazosin (□), WB4101 (■), benoxathian (●), or phentolamine (△). Plotted is a representative experiment (total binding, 883 cpm; nonspecific binding, 504 cpm; specific binding, 489 cpm) that was replicated four to seven times for each antagonist, using different membrane preparations.

TABLE 1

Binding parameters derived from the competition by agonists and antagonists for 5-[^{3}H]methylurapidil binding sites

Results are the means ± standard errors of the number of determinations, using different membrane preparations, indicated in parentheses.

	K_i	Slope
	nM	
Agonists		
Oxymetazoline (4)	27 ± 8	0.61 ± 0.06
(−)-Epinephrine (4)	193 ± 13	0.52 ± 0.05
(−)-Norepinephrine (4)	321 ± 70	0.69 ± 0.05
Methoxamine (4)	3738 ± 587	0.66 ± 0.05
Antagonists		
(+)-Niguldipine (4)	0.55 ± 0.13	0.80 ± 0.06
5-Methylurapidil (4)	1.21 ± 0.50	0.89 ± 0.07
Prazosin (7)	1.34 ± 0.22	0.87 ± 0.08
WB4101 (6)	1.39 ± 0.35	0.85 ± 0.10
Benoxathian (5)	3.08 ± 0.31	0.82 ± 0.10
Phentolamine (5)	9.00 ± 1.70	0.75 ± 0.06
(−)-Niguldipine	13.30 ± 1.30	0.89 ± 0.05

adrenergic responsiveness of hepatocytes isolated from guinea pigs (9, 12) and, consistent with this, it had little effect on the number of sites or their affinity for 5-[^{3}H]methylurapidil (Fig. 2). Preincubation and washing of the membranes alone reduced the number of sites; the explanation for this is not known but it does not seem to be due to receptor proteolysis, because the inclusion of protease inhibitors in the buffer did not prevent the decrease.

(+)-Niguldipine was a very potent antagonist in this model. The K_i observed (0.55 nM) in the present experiments was higher than that observed for the high affinity component in rat brain cortex membranes (0.05 nM) (24), but it was much lower than those observed for the cloned receptors (α_{1C}, 80 nM; α_{1B}, 1700 nM; and $\alpha_{1A/D}$, 46–1100 nM) (6, 7). (−)-Niguldipine was ≈24-fold less potent than its enantiomer, which is similar to what was observed in brain membranes for α_{1A}-adrenergic sites (40-fold difference) (24).

Benoxathian also had relatively high affinity (3 nM) for the α_1-adrenoceptors of guinea pig liver membranes; however, such affinity was lower than that observed in the rat submaxillary gland and the rat cerebral cortex (high affinity component) (25) but much higher than that observed with the cloned receptors ($\alpha_{1A/D}$, 7700 nM; α_{1B}, 2100 nM; and α_{1C}, 500 nM) (7).

Major differences were also observed when the K_i values for agonists of the cloned receptors (7) were compared with those of the guinea pig liver membrane receptors. Oxymetazoline was very potent in these membranes, although it is a partial agonist in whole guinea pig hepatocytes (26).

The number of members of the α_1-adrenoceptor family is currently four, but we cannot eliminate the possibility that other members may exit. It is clear that the pharmacological definition of subtypes is far from easy and that, as discussed for α_2-adrenoceptors (27), at this point it is clearly inappropriate to define a receptor subtype using a single drug. The data discussed above regarding the α_1-adrenoceptors present in guinea pig liver membranes indicate that such receptors are pharmacologically different from the three cloned receptors. These liver membrane receptors are similar to the so-called classical α_{1A}-adrenoceptors, although some differences were also observed. It is possible that such pharmacological differences could exist between species homologues, because amino acid substitution may alter the stereochemical conformation of the binding sites.

Finally, we would like to discuss the fact that we observed expression of the $\alpha_{1A/D}$-adrenoceptors in guinea pig hepatocytes, by Northern analysis using a cDNA probe (9) (from the atypical α_{1A}-adrenoceptor) (5, 7). This may suggest the coexpression of the classical and atypical α_{1A}-adrenoceptors in guinea pig hepatocytes; however, as indicated above we have no evidence for heterogeneity. On the other hand, it is also possible that the sequences of these receptors are so similar that the probe cross-hybridizes even under high stringency conditions. By Northern

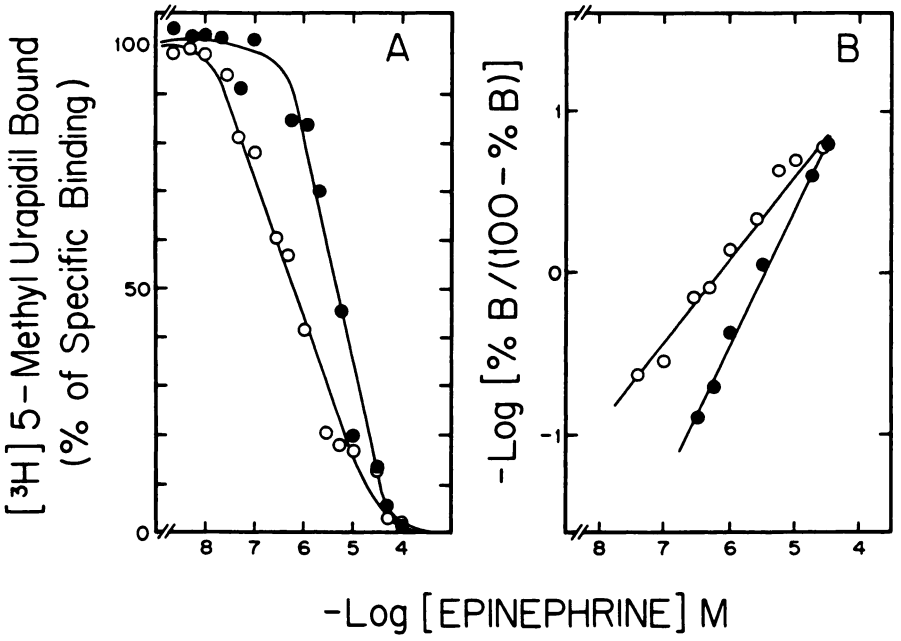

Fig. 6. Effect of Gpp(NH)p on the competition by epinephrine for 5-[^{3}H]methylurapidil binding sites. Binding competition was performed using ≈3 nM 5-[^{3}H]methylurapidil in the absence (○) or presence (●) of 100 μM Gpp(NH)p and varying concentrations of epinephrine. A, Competition for specific binding; B, Hill analysis. Plotted is a representative experiment (total binding, 860 cpm; nonspecific binding, 402 cpm; specific binding, 458 cpm) that was replicated four times using different membrane preparations.

TABLE 2

Effect of Gpp(NH)p on the competition by epinephrine for 5-[^{3}H] methylurapidil binding sites

Results are the means ± standard errors of four determinations, using different membrane preparations.

	Epinephrine	Epinephrine + Gpp(NH)p
Hill coefficient	0.55 ± 0.05	0.89 ± 0.09
LIGAND analysis		
K_H (nM)	28 ± 6	
K_L (nM)	678 ± 96	564 ± 64
R_H (%)	44 ± 8	
R_L (%)	56 ± 8	100

analysis it was observed that the cloned α_{1A} receptor has the tissue distribution expected for the classical α_{1A}-adrenoceptor.

In summary, our data indicate that guinea pig liver membranes have a discrete number of α_1-adrenoceptors that can be studied using 5-[^{3}H]methylurapidil. The pharmacological characteristics of these receptors differ from those of the cloned receptors and show similarities to those of the classical α_{1A}-adrenoceptors. The affinity for agonists of these receptors seems to be modulated by a regulatory G protein.

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References

1. Morrow, L. A., and I. Creese. Characterization of α_1-adrenergic receptor subtypes in rat brain: a reevaluation of [^{3}H]WB4101 and [^{3}H]prazosin binding. *Mol. Pharmacol.* **29**:321–330 (1986).
2. Minneman, K. P. α_1-Adrenergic receptor subtypes, inositol phosphates and sources of cell Ca^{2+}. *Pharmacol. Rev.* **40**:87–119 (1988).
3. Cotecchia, S., D. A. Schwinn, R. R. Randall, R. J. Lefkowitz, M. G. Caron, and B. K. Kobilka. Molecular cloning and expression of the cDNA for the hamster α_1-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* **85**:7159–7163 (1988).
4. Schwinn, D. A., J. W. Lomasney, W. Lorenz, P. J. Szklut, Jr., R. T. Frameau, T. L. Yang-Feng, M. G. Caron, R. J. Lefkowitz, and S. Cotecchia. Molecular cloning and expression of the cDNA for a novel α_1-adrenergic receptor subtype. *J. Biol. Chem.* **265**:8183–8189 (1990).
5. Lomasney, J. W., S. Cotecchia, W. Lorenz, W.-Y. Leung, D. A. Schwinn, T. L. Yang-Feng, M. Brownstein, R. J. Lefkowitz, and M. G. Caron. Molecular cloning and expression of the cDNA for the α_{1A}-adrenergic receptor, the gene for which is located on human chromosome 5. *J. Biol. Chem.* **266**:6365–6369 (1991).
6. Pérez, D. M., M. T. Piascik, and R. M. Graham. Solution-phase library screening for the identification of rare clones: isolation of an α_{1D}-adrenergic receptor cDNA. *Mol. Pharmacol.* **40**:876–883 (1991).
7. Schwinn, D. A., and J. W. Lomasney. Pharmacologic characterization of cloned α_1-adrenoceptor subtypes: selective antagonists suggest the existence of a fourth subtype. *Eur. J. Pharmacol.* **227**:433–436 (1992).
8. Schwinn, D. A., S. O. Page, J. P. Middleton, W. Lorenz, S. B. Liggett, K. Yamamoto, E. G. Lapetina, M. G. Caron, R. J. Lefkowitz, and S. Cotecchia. The α_{1C}-adrenergic receptor: characterization of signal transduction pathway and mammalian tissue heterogeneity. *Mol. Pharmacol.* **40**:619–626 (1991).
9. García-Sáinz, J. A., M. T. Romero-Avila, R. Alcántara-Hernández, M. Macías-Silva, A. Olivares-Reyes, and C. González-Espinosa. Species heterogeneity of hepatic α_1-adrenoceptors: α_{1A}, α_{1B} and α_{1C} subtypes. *Biochem. Biophys. Res. Commun.* **186**:760–767 (1992).
10. Han, C., P. W. Abel, and K. P. Minneman. Heterogeneity of α_1-adrenergic receptors revealed by chlorethylclonidine. *Mol. Pharmacol.* **32**:505–510 (1987).
11. Torres-Márquez, M. E., R. Villalobos-Molina, and J. A. García-Sáinz. α_1-Adrenoceptor subtypes in aorta (α_{1A}) and liver (α_{1B}). *Eur. J. Pharmacol.* **206**:199–202 (1991).
12. García-Sáinz, J. A., M. T. Romero-Avila, J. A. Olivares-Reyes, and M. Macías-Silva. Guinea pig hepatocyte α_{1A}-adrenoceptors: characterization, signal transduction and regulation. *Eur. J. Pharmacol.* **227**:239–245 (1992).
13. Neville, D. M., Jr. Isolation of an organ specific protein antigen from cell surface membrane of rat liver. *Biochim. Biophys. Acta* **154**:540–552 (1968).
14. Munson, P. J., and D. Rodbard. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).
15. Boyer, J. L., A. Garcia, C. Posadas, and J. A. García-Sáinz. Differential effect of pertussis toxin on the affinity state for agonists of renal α_1- and α_2-adrenoceptors. *J. Biol. Chem.* **259**:8076–8079 (1984).
16. Medina-Martinez, O., and J. A. García-Sáinz. Hepatocyte homologous β_2-adrenergic desensitization is associated with a decrease in number of plasma membrane β_2-adrenoceptors. *Eur. J. Pharmacol.* **244**:145–151 (1993).
17. Cheng, Y.-C., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of an inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
19. De Lean, A., J. M. Stadel, and R. J. Lefkowitz. A ternary complex model

explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor. *J. Biol. Chem.* **255**:7108–7117 (1980).
20. Samama, P., S. Cotecchia, T. Costa, and R. J. Lefkowitz. A mutation-induced activated state of the β_2-adrenergic receptor: extending the ternary complex model. *J. Biol. Chem.* **268**:4625–4636 (1993).
21. Laporte, A.-M., L. E. Schechter, F. J. Bolaños, D. Vergé, M. Hamon, and H. Gozlan. [^{3}H]5-Methyl-urapidil labels 5-HT_{1A} receptors and α_1-adrenoceptors in the rat CNS: *in vitro* binding and autoradiographic studies. *Eur. J. Pharmacol.* **198**:59–67 (1991).
22. Graziadei, I., G. Zernig, R. Boer, and H. Glossman. Stereoselective binding of niguldipine enantiomers to α_{1A}-adrenoceptors labeled with [^{3}H]5-methyl-urapidil. *Eur. J. Pharmacol.* **172**:329–337 (1989).
23. Hanft, G., and G. Gross. Subclassification of α_1-adrenoceptor recognition sites by urapidil derivatives and other selective antagonists. *Br. J. Pharmacol.* **97**:691–700 (1989).
24. Boer, R., A. Grassegger, C. Schudt, and H. Glossmann. (+)-Niguldipine binds with very high affinity to Ca^{2+} channels and to a subtype of α_1-adrenoceptors. *Eur. J. Pharmacol.* **172**:131–145 (1989).
25. Michel, A. D., D. N. Loury, and R. L. Whiting. Identification of a single α_1-adrenoceptor corresponding to the α_{1A}-subtype in rat submaxillary gland. *Br. J. Pharmacol.* **98**:883–889 (1989).
26. García-Sáinz, J. A., M. T. Romero-Avila, R. Alcántara-Hernández, and A. Olivares-Reyes. Different sensitivity to methoxamine and oxymetazoline of hepatocytes expressing α_{1A}-, α_{1B}- or α_{1C}-adrenoceptors. *Pharmacol. Commun.* **2**:339–344 (1993).
27. Bylund, D. B., H. S. Blaxall, L. J. Iversen, M. G. Caron, R. J. Lefkowitz, and J. W. Lomasney. Pharmacological characteristics of α_2-adrenergic receptors: comparison of pharmacologically defined subtypes with subtypes identified by molecular cloning. *Mol. Pharmacol.* **42**:1–5 (1992).

Send reprint requests to: J. Adolfo García-Sáinz, Instituto de Fisiología Celular, UNAM, Apartado Postal 70–248, 04510 México DF, México.