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HYPOTENSIVE ACTIVITY OF SEROTONIN ANTAGONISTS; CORRELATION WITH α_1-ADRENOCEPTOR AND SEROTONIN RECEPTOR BLOCKADE

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Summary

For a series of 12 serotonin antagonists, largely varying in potency, the decrease in diastolic pressure was determined after intravenous injection into pentobarbitone-anaesthetized normotensive rats. The hypotensive activity of these antagonists was correlated with their affinity for α_1-adrenoceptors, established by (^{3}H)prazosin radioligand displacement, and the 5-HT_2 serotonergic receptor, determined by inhibition of specific (^{3}H)mianserin binding. The radioligand binding assays were performed since they correspond to the *in vivo* antagonistic potencies of the antagonists at α_1- and 5-HT_2-receptors, respectively. A close correlation ($r = 0.963$) was found between the affinity for α_1-adrenoceptors and hypotensive activity. On the other hand, a negative correlation of lower statistical quality ($r = -0.808$) existed between the affinity for 5-HT_2-receptors and the depressor potency. In this series of 12 compounds, the new antihypertensive drug ketanserin is included for which it has been speculated that it lowers blood pressure by virtue of its serotonin antagonistic activity. The results of the present study, however, point towards α_1-adrenolytic potency as an important mechanism in the hypotensive action of the drug.

Recently, a new antihypertensive drug has been introduced for which an essentially new mode of action has been proposed. This drug, ketanserin (Janssen Pharmaceutica, Belgium), inhibits serotonin-induced vasoconstriction (1) and abolishes the amplifying effect of serotonin on noradrenaline-evoked vasoconstriction (1) as well as noradrenaline-evoked platelet aggregation (2). It is suggested that ketanserin lowers arterial pressure by virtue of its serotonin antagonistic potency (3,4). However, firm evidence for this putative new approach to the drug treatment of hypertension is as yet not available.

Clinical experience with ketanserin points to a marked resem-

blance with prazosin (4). In fact, when α-adrenoceptors only are considered, ketanserin, like prazosin, is a selective antagonist of the α_1-adrenoceptor subtype (5,6). By using a series of 12 antagonists, largely varying in their potency to inhibit α_1- and/or serotonin induced vasoconstriction, attempts were made in the present paper to answer the question whether serotonin antagonism can be held responsible for a hypotensive effect or simply the α_1--adrenoceptor blocking activity.

Materials and Methods

Hypotensive effect in pentobarbitone-anaesthetized rats: Male, normotensive Wistar rats of 200-250 g were used. Anaesthesia was induced by an intraperitoneal injection of pentobarbitone-sodium in a dose of 75 mg/kg of body weight. The trachea was cannulated and artificial respiration with room air was initiated. The right jugular vein and ipsilateral common carotid artery were cannulated for drug administration and blood pressure monitoring, respectively. Arterial blood pressure and pulse rate were established by means of a Hellige HE-19 device via a Statham P23 Db pressure transducer. At least three different doses of the antagonists were used to calculate the dose that caused 30% reduction in diastolic pressure (EC_{30}, in mol/kg). Each dose of the antagonist was administered in a volume of 1 ml/kg to at least six different animals. Only those animals with a diastolic pressure within the range 100--130 mm Hg were included in the study.

Affinity for (central) α_1-adrenoceptors: Cerebral membranes obtained from male normotensive Wistar rats (200-250 g) were prepared as described in (7) and (8). In short, the animals were decapitated and their brains (minus cerebella) were homogenized in 20% w/v of ice-cold Tris/HCl buffer (pH = 7.7). After work-up as usual the suspension was adjusted to contain a protein concentration of 1 mg/ml. Aliquots of 500 µl were incubated at 25°C for about 60 min with [^{3}H]prazosin (specific activity 33 Ci/mmol; 0.2 nM) and various concentrations of non-radioactive competitors in a total volume of 1 ml. The specific binding of [^{3}H]prazosin was defined as the excess over blanks containing 2 µM phentolamine. Incubations were terminated by rapid vacuum filtration through Whatman GF/B filters. Filters were washed by three 5-ml portions of ice-cold Tris/HCl buffer, left to solubilize in 10 ml Hydrocount for 24 h and counted for radioactivity at 35-40% efficiency. Log probit analysis was applied to linearize the displacement plots obtained and to calculate the IC_{50}-values.

Affinity for 5-HT_2-receptors in rat brain tissue: Selective labeling of 5-HT_2-receptors was performed using the method of Peroutka and Snyder (9). In brief, after decapitation of the rats, brains were rapidly removed and frontal cortices were dissected on ice. The tissue was homogenized in ice-cold Tris/HCl buffer (pH = 7.7) and worked up as usual. Incubation tubes contained 0.2 ml [^{3}H]-mianserin (10^{-9} M), 0.5 ml tissue suspension (2 mg protein/ml), 0.1 ml 3 x 10^{-6} M triprolidine and 0.2 ml of the various antagonistic drugs, respectively. After incubation at 25°C for approximately 45 min, labeled membranes were collected by rapid vacuum filtration through Whatman GF/B filters and subsequently washed with three 4 ml rinses of ice-cold Tris/HCl buffer. The filters were transfered to glass counting vials and left to solubilize in 10 ml of Hydrocount for 24 h at room temperature and finally counted at an effi-

ciency of 35-40%. Specific binding of (^{3}H)mianserin was defined as the excess over blanks containing 1 µM of cyproheptadine.

Drugs used: The following substances were used (sources in parentheses): prazosin HCl and its analogue UK 33,274 (Pfizer); WB 4101 (Ward Blenkinsop); phentolamine HCl (Ciba); ketanserin (Janssen Pharmaceutica); metergoline (Pharmitalia); cyproheptadine HCl (M.S.D.); cinanserin HCl (Squibb); methysergide maleate (Sandoz); (+)-mianserin and (-)-mianserin (Organon); metitepine maleate (Hoffmann-La Roche); (^{3}H)mianserin and (^{3}H)prazosin (New England Nuclear); Hydrocount^R (Baker) and pentobarbitone-sodium (Merck). WB 4101, phentolamine and metitepine were dissolved in saline; prazosin and UK 33,274 in 10% w/v glucose solution; ketanserin and the mianserin isomers in 0.02 M tartaric acid solution; metergoline, cyproheptadine, cinanserin and methysergide in a mixture of 3 parts 96% ethylalcohol and 7 parts of 10% w/v glucose solution.

Statistical evaluation: Plots of log dose antagonist (mol/kg) versus percentage decrease in diastolic pressure were considered linear. EC_{30}-values were calculated by linear regression analysis and expressed as mol/kg. The slopes of these plots were used to explore parallelism (10). Regression analysis was applied to judge the relationship between receptor affinity and hypotensive activity.

Results

Following the intravenous injection into anaesthetized normotensive rats, all of the antagonists decreased diastolic pressure in a dose dependent manner (fig. 1). Where ethanol was used to dissolve the antagonistic drug, the hypotensive activity of the solvent itself could always clearly be distinguished from the (longer lasting) effect of the hypotensive drug. The other solvents were inactive. The slopes of the log dose/hypotensive ef-

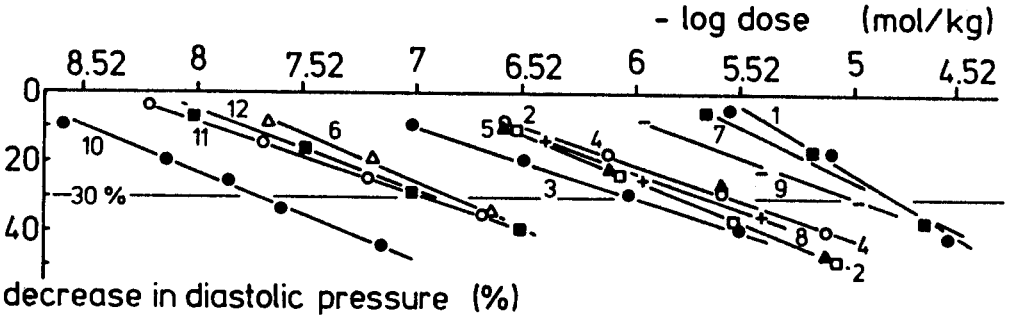

FIG. 1

Dose-dependent decrease in diastolic pressure of pentobarbitone-anaesthetized normotensive rats, brought about by 12 serotonin antagonists, largely varying in potency. Numbers refer to the compounds listed in table 1.

fect plots did not differ significantly ($p > 0.05$). Doses (mol/kg) that caused a 30% reduction in diastolic pressure, expressed as pEC_{30}, are listed in table 1.

TABLE 1

Hypotensive Activities (pEC_{30}; EC_{30} in mol/kg) Following Intravenous Injections into Anaesthetized Normotensive Rats and Affinities for [^{3}H]Prazosin-labeled α_1-, -log IC_{50} α_1, and [^{3}H]Mianserin-identified 5-HT_2-receptors, -log IC_{50} 5-HT_2, in Rat Cerebral Membranes. The Values Listed have been Employed to Derive the Equations 1 and 2.

No.	Drug	pEC_{30}	-log IC_{50} α_1	-log IC_{50} 5-HT_2
1.	Methysergide	4.85	5.23	8.49
2.	Cyproheptadine	5.83	6.61	8.43
3.	Phentolamine	5.98	7.41	6.62
4.	Ketanserin	5.55	7.16	8.32
5.	Metergoline	5.63	6.61	8.39
6.	Metitepine	6.87	8.86	7.89
7.	Cinanserin	4.86	5.20	8.49
8.	(+)-Mianserin	5.71	6.58	8.43
9.	(-)-Mianserin	5.00	5.65	7.46
10.	Prazosin	7.78	9.25	3.80
11.	UK 33,274	6.96	8.06	4.42
12.	WB 4101	6.94	8.15	5.90

The ability of the antagonists to displace the selective binding of [^{3}H]mianserin and [^{3}H]prazosin were expressed as -log IC_{50}. The values are listed in table 1.
The following equations were derived:

$$pEC_{30} = 0.68\ (-\log IC_{50\,\alpha_1}) + 1.21 \qquad (1)$$
$$n = 12 \quad r = 0.963 \quad s = 0.267 \quad F = 128$$

$$pEC_{30} = 0.46\ (-\log IC_{50\,5\text{-}HT_2}) + 9.28 \qquad (2)$$
$$n = 12 \quad r = -0.808 \quad s = 0.584 \quad F = 19$$

Equation (1) reveals a close correlation between hypotensive activity and α_1-adrenoceptor affinity. Equation (2) possessing less statistical meaning than equation (1) shows that the depressor potency is negatively correlated with 5-HT_2 receptor affinity.

Discussion

Numerous reports describe an amplifying effect of serotonin (5-hydroxytryptamine; 5-HT) on vasoconstrictor responses to noradrenaline. This potentiating effect seems a general phenomenon, since it is observed in isolated vessels of various species, including man (1,11-15), as well as under *in vivo* circumstances in the dog (16,17). The transport of serotonin with circulating blood occurs by the thrombocytes. Subthreshold concentrations of serotonin amplify the platelet-aggregating activity of noradrenaline in human platelet-rich plasma (2). Thrombocytes release their serotonin content when they aggregate.
It is suggested that serotonin by its own vasoconstrictor response

and by the amplification of the effect of noradrenaline (and possibly other vasoactive compounds) might play a part in blood pressure regulation or might even be involved in the pathogenesis of essential hypertension (3,4). According to this view serotonin antagonists should be considered potential antihypertensive drugs (especially in atherosclerotic patients). However, compounds that lower blood pressure by virtue of their serotonin antagonistic potency have not been described so far. Presently a new drug (ketanserin, Janssen Pharmaceutica, Belgium) has been introduced, for which the antihypertensive activity is claimed to be due to the specific blockade of vascular 5-HT_2-receptors (1). Ketanserin indeed is a potent 5-HT_2 antagonist (1,5,6), it is devoid of affinity for 5-HT_1 receptors (18) and devoid of agonistic effects (1). The drug exhibits a 10-fold less affinity for α_1- and H_1-receptors (18). At present ketanserin is used as a tool to study a possible pathophysiological role of serotonin in the pathogenesis and maintenance of essential hypertension (3,4). Conversely, the inhibition of serotonin-evoked vasoconstriction and the abolition of the amplifying effect of serotonin (on vessels and platelets) by ketanserin is regarded as the basis of the mode of action by which this drug lowers blood pressure (1,2,19). Before one can introduce a new category of antihypertensive drugs without the existence of previous evidence that 5-HT receptors are involved in the maintenance of the vascular tone, an answer has to be given to the question why ketanserin would be unique among other serotonin antagonists, such as cyproheptadine, methysergide, mianserin, BW 501 C67, etc. Among the potent 5-HT_2-antagonists ketanserin is the most selective drug with respect to the ratio 5-HT_1/5-HT_2 receptor affinity (18). Binding to the 5-HT_1 receptor cannot be related to any biological effect of serotonin (20). The relevance of the absence of affinity for this serotonin receptor therefore remains questionable. Ketanserin is, unlike methysergide or ergotamine, devoid of intrinsic activity (1), but so are cyproheptadine, mianserin, BW501C67, etc. In rats, ketanserin in hypotensive doses inhibited the vasoconstriction due to the selective α_1-antagonist methoxamine (6) and the α_1-adrenoceptor mediated hypertensive response to electrical stimulation of the spinal cord (5). BW501C67, a serotonin antagonist almost without affinity for α-adrenoceptors is ineffective in lowering blood pressure (5). For these reasons one is tempted to believe that the α_1-adrenoceptor blocking activity of ketanserin would be underestimated with respect to the drug's hypotensive potency.

In the present experiments α_1-adrenoceptor blocking and 5-HT_2-antagonistic potency were established by means of *in vitro* radioligand displacement studies using cerebral membranes. The *in vitro* affinity for central α_1-adrenoceptors has been shown to correspond to the α_1-adrenolytic activity in the vascular system of the rat (21). Moreover, *in vitro* affinity for rat frontal cortex 5-HT_2 receptors has been shown to correlate with the antagonistic potency towards serotonin-induced vasoconstriction in the pithed rat (Kalkman, submitted for publication). The group of antagonists studied was selected in such a way that the compounds largely differed in their α_1- and 5-HT_2 antagonistic activity. The correlation equation (1) indicates that 93% (= r^2) of the variance in blood pressure decreasing activity can be explained by the α_1-sympatholytic potency. In contrast, only a negative relationship with fair statistics, was established between the blood pressure decreasing activity and the serotonin antagonistic potency. This

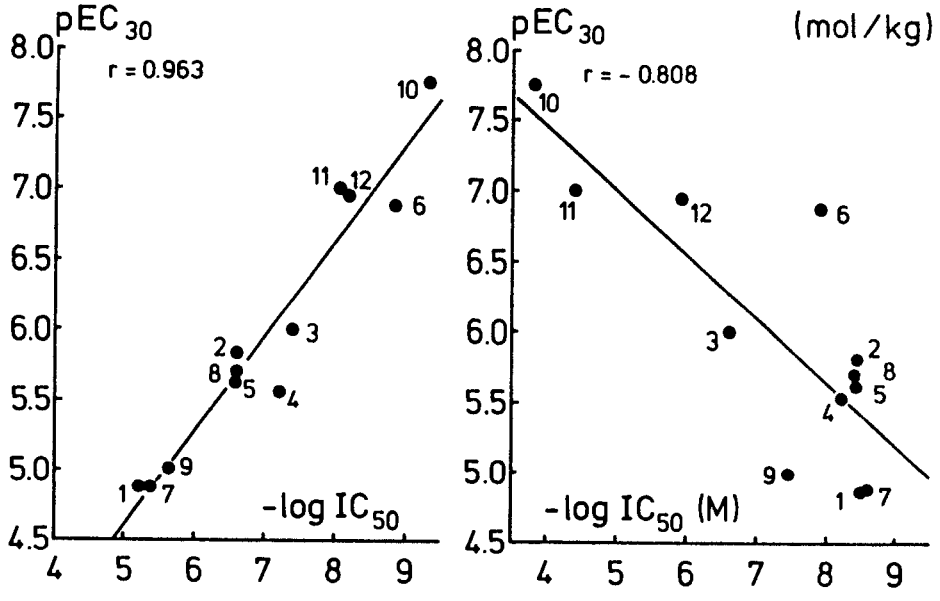

FIG. 2

Correlation between hypotensive activity of twelve serotonin antagonists (expressed as pEC_{30} values, see table 1) and their potency to displace specific (^3H) prazosin binding (expressed as -log IC_{50} in the left-hand pannel) and specific (^3H)mianserin binding (right-hand pannel). Numeric values of -log IC_{50} and the numbers of the compounds are listed in table 1.

finding most probably reflects the selectivity of the serotonin antagonists studied with respect to the α_1-adrenoceptor.

The outcome of the present study provides strong indication that it is rather the α_1-adrenoceptor blocking activity than the 5-HT_2 receptor antagonistic potency of the drugs which is related to the blood pressure lowering activity, as determined in anaesthetized normotensive rats. This result gives further support to earlier observations (5,6) that ketanserin diminishes arterial pressure of rats by a functional blockade of vascular α_1-adrenoceptors.

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References

1. J.M. van NUETEN, P.A.J. JANSSEN, J. van BEEK, R. XHONNEUX, T.J. VERBEUREN and P.M. VANHOUTTE, J. Pharmacol. Exp. Ther., 218, 217-230 (1981).
2. J.M. van NUETEN, F. de CLERK, R. XHONNEUX and P.M. VANHOUTTE, Abstract 425, VIII Int. Congr. Pharmacol. Proc. Tokyo (1981).
3. J. de CRÉE, J. LEEMPOELS, H. GEUKENS, W. de COCK and H. VERHAEGEN, Clin. Sci. 61, 473s-476s (1981).
4. G.J. WENTING, A.J. MAN IN 'T VELD, A.J. WOITTIEZ, F. BOOMSMA and M.A.D.H. SCHALEKAMP, Brit. Med. J. 284, 537-539 (1982).
5. J.R. FOZARD, J. Cardiovasc. Pharmacol., 4, 829-838 (1982).
6. H.O. KALKMAN, P.B.M.W.M. TIMMERMANS and P.A. van ZWIETEN, J. Pharmacol. Exp. Ther. 222, 227-231 (1982).
7. D.A. GREENBERG, D.C. U'PRICHARD and S.H. SNYDER, Life Sci., 19, 69-76 (1976).
8. D.C. U'PRICHARD, D.A. GREENBERG and S.H. SNYDER, Mol. Pharmacol. 13, 454-473 (1977).
9. S.J. PEROUTKA and S.H. SNYDER, J. Pharmacol. Exp. Ther. 216, 142-148 (1981).
10. R.J. TALLARIDA and L.S JACOB, The Dose-Response Relation in Pharmacology , Springer, Berlin-Heidelberg-New York (1979).
11. I.S. de la LANDE, V.A. CANNELL and J.G. WATERSON, Brit. J. Pharmac. Chemother. 28, 255-272 (1966).
12. P.R. CARROLL, P.W. EBELING and W.E. GLOVER. Austr. J. Exp. Biol. Med. 52, 813-823 (1974).
13. J.R. FOZARD, Eur. J. Pharmacol. 36, 127-139 (1976).
14. R.F.W. MOULDS and P.J. WORLAND, J. Cardiovasc. Pharmacol. 2, 377-386 (1980).
15. H. MORITOKI and C. SU, Arch. Int. Pharmacodyn. 252, 186-195 (1981).
16. R. HURWITZ, R.W. CAMPBELL, P. GORDON and F.J. HADDY, J. Pharmacol. Exp. Ther. 133, 57-62 (1961).
17. A.A. MARTINEZ and M.F. LOKHANDWALA, Eur. J. Pharmacol. 63, 303-311 (1980).
18. J.E. LEYSEN, F. AWOUTERS, L. KENNIS, P.M. LADURON, J. VANDENBERK and P.A.J. JANSSEN, Life Sci. 28, 1018-1022 (1981).
19. J.M. van NUETEN, P.A.J. JANSSEN, W. de RIDDER and P.M. VANHOUTTE, Eur. J. Pharmacol.77, 281-287 (1982).
20. S.J. PEROUTKA, R.M. LEBOWITZ and S.H. SNYDER, Science 212, 827-829 (1981).
21. P.B.M.W.M. TIMMERMANS, F. KARAMAT ALI, H.Y. KWA, A.M.C. SCHOOP, F.P. SLOTHORST-GRISDIJK and P.A. van ZWIETEN, Mol. Pharmacol. 20, 295-301 (1981).