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# Correlation between the affinity for [$^3$H]mianserin-labelled receptors in brain and antagonism of the serotonin pressor response in pithed rats

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Two distinct types of serotonin receptor binding sites have been identified in *in vitro* binding studies [1–3]. One class, the $S_1$-receptor, is specifically labelled by tritiated serotonin. Frontal cortex tissue contains the so-called $S_2$-receptor [2, 3], which was originally defined by the high affinity binding of [$^3$H]spiperone. At present two other ligands, [$^3$H]mianserin and [$^3$H]ketanserin, are available to investigate the affinity for the $S_2$-receptor. They are advocated to be more suitable for use as the $S_2$-ligand than [$^3$H]spiperone [4, 5].

The subdivision of serotonin receptors is of physiological relevance since the $S_2$-receptor, in contrast to the $S_1$-receptor, is associated with serotonin-induced blood vessel contraction [6], as assayed *in vitro*. Affinity for brian $S_2$-receptors, identified by [$^3$H]ketanserin, has been found to correspond with the antagonistic activity of numerous compounds towards serotonin-evoked vasoconstriction of the rat caudal artery [5]. A study of serotonin-mediated vasoconstriction *in vitro* has the advantage that the results are not affected by pharmacokinetic factors. This approach, however, has one major drawback—that the vessel which can be used in such an assay does not determine the peripheral resistance in an *in vivo* situation.

The objective of the present study was to investigate whether the affinity for brain $S_2$-receptors could be correlated with the inhibitory activity of antagonists towards the vasopressor effect of serotonin in the intact circulatory system of pithed rat. Affinity for brain $S_2$-receptors was determined by the method of Peroutka and Snyder [4] using [$^3$H]mianserin.

## *Materials and methods*

*Animals.* Male Wistar normotensive rats (200–250 g) were used throughout the study. The animals had been kept at a standardized diet of normal chow (Muracon), and water *ad lib.*

*Radioligand binding assay.* Selective labelling of $S_2$-receptors was performed using the method of Peroutka and Snyder [4]. In brief, after decapitation, brains were quickly removed and the frontal cortex was dissected on ice. This tissue was homogenized in ice-cold Tris–HCl buffer (pH 7.7). The homogenate was centrifuged at 50,000 *g* for 10 min, and washed twice with buffer. If necessary the pellet was stored at −20°. Incubation tubes contained 0.2 ml of $10^{-9}$ M [$^3$H]mianserin, 0.5 ml tissue suspension containing 2 mg protein/ml, 0.1 ml of $3 \times 10^{-6}$ M triprolidine and a 0.2 ml solution of the drug to be tested. All assays were performed in triplicate. After incubation at 25° for 45 min, labelled membranes were collected by rapid vacuum filtration through Whatman GF/B filters and washed with three 4 ml rinses of ice-cold Tris–HCl buffer. The filters were transferred to glass counting bottles and left to solubilize in 10 ml Hydrocount® (Baker Chemicals, Philipsberg, PA) for 24 hr at room temperature and finally counted at an efficiency of about 40%. Specific binding of [$^3$H]mianserin was defined as the excess over blanks containing 1 μM cyproheptadine. Specific binding accounts for approximately 60% of total binding at a concentration of 0.2 nM, the amount of [$^3$H]mianserin used in routine drug competition studies.

*Antagonism towards serotonin* in vivo. When administered i.v. to pithed normotensive male Wistar rats, serotonin evokes a dose-dependent pressor response [7]. This increase in blood pressure is selectively inhibited by serotonin antagonists [7, 8]. $ED_{50}$ values for antagonistic potency of drugs were determined as follows. Pithed rats received an i.v. control dose of 100 μg/kg serotonin as a bolus injection. Pre-treatment (i.v.) with a low dose of antagonist was started and 15 min later serotonin (100 μg/kg) was re-administered. As soon as the diastolic pressure passed its maximal value, another appropriate additive amount of antagonist was given, and 15 min later serotonin was injected again. This procedure was repeated once. Each animal therefore received three doses of antagonist in a cumulative order (e.g. 1 + 2 + 7 mg/kg). Control experiments had shown that in the course of 90 min the repeated administration of 100 μg/kg serotonin at 15 min intervals elicited identical pressor responses. The increases in diastolic pressure observed after the administration of antagonist were transformed into percentages of the control response. Regression analysis of these values against the respective log dose of the antagonist yielded the $ED_{50}$ value, i.e. the dose (mole/kg) of antagonist causing a reduction of the initial pressor responses to 100 μg/kg serotonin by 50%. At least six different rats were used for one antagonist.

*Drugs.* [$^3$H]mianserin (55 Ci/mmole) was purchased from New England Nuclear (Boston, MA). The ligand was stored at −20° and diluted in Tris–HCL buffer to a concentration of $10^{-9}$ M immediately before use. Unlabelled drugs were

either gifts by the manufacturers of origin or were purchased from Sigma Chem. Co. (St. Louis, MO).

*Mathematical analysis.* $IC_{50}$ values (M) derived from displacement curves were determined by log probit analysis [9]. Methods and formulae applied to calculate regression lines, 95% confidence limits and regression coefficients have been described before in full detail [9].

*Results*

A dose of 100 μg/kg of serotonin was chosen because it evoked an increase in diastolic pressure in pithed normotensive rats by about 80% of the maximally obtainable response, while repeated administration of this dose at 15 min intervals showed no tachyphylaxis during a 90 min test period. A typical example of an $ED_{50}$ calculation is shown in Fig. 1. Within the group of 11 antagonists studied, metabolic breakdown during the experiment could be ignored since no significant differences ($P > 0.05$) were observed between the increase in diastolic pressure after single administration of the highest dose or after addition of the drug in steps (see Materials and Methods).

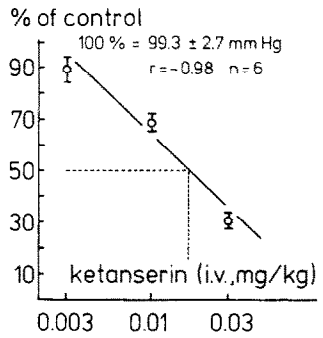


Fig. 1. A typical example of the determination of the serotonin antagonistic potency quantified as an $ED_{50}$ value. Percentages of the control value of the increase in diastolic pressure of pithed normotensive rats evoked by 100 μg/kg i.v. serotonin (ordinate) were plotted against the corresponding log dose of the serotonin antagonist (abscissa). The dose of serotonin antagonist that reduced the vasopressor response to serotonin by 50% ($ED_{50}$ value) was calculated by linear regression analysis.

Within the group of 11 serotonin antagonists, a close correlation ($r = 0.89$) was demonstrated to exist between the following parameters: (i) the negative logarithm of the dose (mole/kg) which caused a 50% reduction of the hypertensive response in pithed normotensive rats ($-\log ED_{50}$); and (ii) the negative logarithm of the molar concentration which caused a 50% displacement of the specific binding of [$^3$H]mianserin ($-\log IC_{50}$). The values are listed in Table 1.

The following equation was derived (see Fig. 2):

$$(-\log ED_{50}) = 1.67\ (\pm 0.64)\ (-\log IC_{50}) - 9.24.$$

The close correlation between both parameters indicates that displacement of [$^3$H]mianserin binding can be used to describe the serotonin antagonistic potency in the vascular system of the pithed rat.

*Discussion*

The objective of the present study was to investigate whether the affinity of compounds for brain $S_2$-receptors could be correlated with their inhibitory activity towards the vasopressor effects of serotonin *in vivo* (pithed rat). Such a correlation would implicate that displacement of the tritiated $S_2$-ligand can be used to describe serotonin antagonist potency in the vascular system of this animal species.

Table 1. Inhibitory potency of various serotonin antagonists with respect to specific [$^3$H]mianserin binding in rat frontal cortex tissue ($-\log IC_{50}$) and the antagonism towards the serotonin-induced vasopressor effect *in vivo* ($-\log ED_{50}$)

| | $-\log IC_{50}$ (M) | $-\log ED_{50}$ (mol/kg) |
|---|---|---|
| 1 ketanserin | 8.32* | 4.40 |
| 2 metitepine | 7.89* | 4.49 |
| 3 cyproheptadine | 8.43* | 4.76 |
| 4 (±)-mianserin | 8.17 | 4.67 |
| 5 methysergide | 8.49* | 5.59 |
| 6 spiperone | 8.32 | 5.12 |
| 7 haloperidol | 7.15 | 2.26 |
| 8 phentolamine | 6.62* | 1.86 |
| 9 cinanserin | 8.49* | 3.72 |
| 10 (+)-mianserin | 8.43* | 4.53 |
| 11 (−)-mianserin | 7.46* | 3.09 |

* Data taken from [13].

Inhibition of the specific binding of [$^3$H]mianserin was determined with nine concentrations of competing drugs in triplicate. The concentration (molar) that displaced the specific binding of the tritiated ligand by 50% ($IC_{50}$) was calculated by log probit analysis. $-\text{Log } IC_{50}$ values were used in the correlation. Antagonism *in vivo* of serotonin-induced increases in diastolic pressure of pithed normotensive rats was assessed as described in Materials and Methods, and quantified as ($-\log ED_{50}$) values.

It is now well established that [$^3$H]mianserin binds with high affinity to $S_2$-receptors [4, 10–12]. In addition, [$^3$H]mianserin binds to histamine $H_1$-receptors [4]. The binding to $H_1$- and $S_2$-receptors can, however, be differentiated by pharmacological and anatomical manipulation [4]. According to Peroutka and Snyder [4], [$^3$H]mianserin can be used to label $S_2$-receptors when rat frontal cortex tissue is taken, and provided that $H_1$-receptors are blocked by triprolidine.

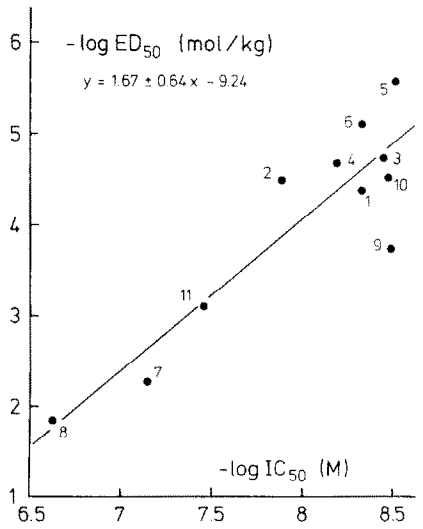


Fig. 2. Correlation between $-\log IC_{50}$ of [$^3$H]mianserin displacement and $-\log ED_{50}$ for the antagonism towards the pressor effect of serotonin *in vivo*. The serotonin antagonistic potency of 11 drugs was determined *in vivo* in the vascular system of the pithed rat by repeated i.v. doses of serotonin before and after increasing doses of the antagonist (see Materials and Methods). $S_2$-Receptors in rat frontal cortex tissue were selectively labelled in a [$^3$H]mianserin binding assay. The various numbers refer to the compounds listed in Table 1.

The finding that a correlation between brain $S_2$-receptor affinity and serotonin antagonist activity *in vivo* exists in the vascular system of the pithed rat makes it likely that serotonin receptors mediating contraction of the resistance vessels belong to the $S_2$-subtype. This study is, however, not decisive on this point. The assumption that increases in diastolic pressure reflect increases in peripheral resistance (and thereby constriction of the arteriolae) is only allowed when cardiac output is constant. This criterion is not met in this study, since it is as yet unknown how different doses of a serotonin antagonist affect cardiac output changes to a single serotonin dose and whether different antagonists behave in the same way in this respect. What can be concluded from this study is that the receptors mediating the hypertensive response to serotonin in the vascular system of the rat belong to the $S_2$-subclass. Thus, affinity for brain $S_2$-receptors can be used to describe the serotonin antagonist potency of a drug towards the hypertensive response to this autocoïd *in vivo*.

In summary, the relationship between binding affinity for $S_2$-receptors in rat frontal cortex tissue assayed *in vitro* using [$^3$H]mianserin and functional antagonism towards pressor effects *in vivo* in the vascular system of the pithed normotensive rat was investigated. A close correlation ($r = 0.89$) was found to exist between both parameters. The vasopressor response to serotonin in the pithed rat is therefore mediated via $S_2$-serotonergic receptors.

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# Selective mitochondrial activity distinguishes aspirin from salicylate and benzoate in yeast cells

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There are numerous reports of the ability of salicylate drugs to inhibit a wide range of metabolic activities in mammalian systems, and attempts are made to correlate these effects with therapeutic properties. Of particular interest to us are the claims that this group of drugs depresses mitochondrial ATP synthesis [1, 2]. It is difficult to establish whether or not the mitochondrial system is solely or selectively affected by a drug in animal cells, but in yeast cells this point can be readily ascertained [3]. The procedure exploits the ability of yeast cells to grow and divide in the absence of mitochondrial respiratory activity (e.g. under anaerobiosis), meeting energy requirements solely from glycolysis. This necessitates the presence of a fermentable energy source such as glucose but if the substrate is non-fermentable (e.g. a Krebs cycle intermediate), then in the absence of respiration, growth would be precluded. By the same token, selective or specific inhibition, either of mitochondrial function or biogenesis, by a drug would not significantly affect growth in glucose medium but would arrest growth in non-fermentable medium. If the anti-mitochondrial activity of the drug in question were due to interaction with the mitochondrial genome (mtDNA), this could induce the mitochondrial mutation known as *petite colonie*. Scoring this condition is greatly facilitated by its exceptionally high spontaneous mutation rate [about 1% of cells of most yeast strains (*Saccharomyces*), give rise to *petite* colonies on plating] and its easily recognisable phenotype of small white colony and respiratory deficiency (for a detailed account of the *petite* mutation, see [4]). Using this yeast system, a comparative study was made of the activities of the chemically related compounds acetyl salicylate (aspirin), salicylic acid and benzoic acid.

## *Materials and methods*

Aspirin (*o*-acetylsalicylic acid) and benzoic acid were obtained from BDH (Dorset, U.K.), and salicylic acid from Sigma Chemical Co. (St. Louis, MO). Eighteen haploid yeast strains of this laboratory were used and culture medium contained 1% yeast extract with 2% glucose (YED) or 4% glycerol (YEG) as fermentable and non-fermentable carbon and energy sources, respectively. Effects on growth by the drugs were assessed first on agar