role, leading to membrane stabilization. At higher concentrations lytic interaction of the drugs with membrane lipids promotes hypotonic haemolysis in red cells.

Besides the drugs tested in this study, similar results were obtained previously for phenothiazine derivatives [12] and propranolol [13]. It seems, therefore, that the above proposed mechanism of drug-induced stabilization and destabilization of biological membranes may be applicable to all cationic local anaesthetics. This model does not account, however, for the action of all antihaemolytic compounds. Fatty acids and some hashish components have been shown to stabilize both erythrocytes and liposomes [12] indicating that direct lipidic interactions are responsible for the antihaemolytic effects of these substances.

In summary, the local anaesthetics dibucaine, tetracaine and procaine increase the osmotic fragility of multilamellar liposomes. Comparison of the effects of these drugs on red cells and model lipid membranes suggests that the crucial role in local anaesthetics-induced stabilization of biological membranes is played by the protein component.

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REFERENCES

1. D. Papahadjopoulos, *Biochim. biophys. Acta* **265**, 169 (1972).
2. G. Strichartz, *Anaesthesiology* **45**, 421 (1976).
3. G. Poste, D. Papahadjopoulos, K. Jacobson and W. J. Vail, *Biochim. biophys. Acta* **394**, 520 (1975).
4. D. Papahadjopoulos, K. Jacobson, G. Poste and G. Shepherd, *Biochim. biophys. Acta* **394**, 504 (1975).
5. M. J. Neal, K. W. Butler, C. F. Polnaszek and I. C. P. Smith, *Molec. Pharmac.* **12**, 144 (1976).
6. A. G. Lee, *Biochim. biophys. Acta* **448**, 34 (1976).
7. J. S. Puskin and T. Martin, *Molec. Pharmac.* **14**, 454 (1978).
8. A. W. De Bruijne and J. van Steveninck, *Biochem. Pharmac.* **26**, 779 (1977).
9. S. Roth and P. Seeman, *Nature, New Biol.* **231**, 284 (1971).
10. P. Seeman, *Pharmac. Rev.* **29**, 583 (1972).
11. W. S. Singleton, M. S. Gray, M. L. Brown and J. L. White, *J. Am. Oil chem. Soc.* **42**, 53 (1965).
12. E. Alhanaty and A. Livne, *Biochim. biophys. Acta* **339**, 146 (1974).
13. W. K. Surewicz, I. Fijalkowska and W. Leyko, *Biochem. Pharmac.* **30**, 839 (1981).

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Comparative effects of ketanserin, a novel serotonergic receptor antagonist, on 5HT-induced shape change and 5HT uptake in rat and human platelets

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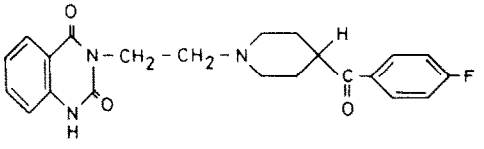
The serotonergic receptor antagonist 3-{2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl}-2,4-(1H, 3H) quinazolinedione, ketanserin (see structure below), has recently



been characterized [1–4]. It antagonizes 5-hydroxytryptamine (5-HT)-induced effects in several pharmacological tests [5, 6]. In particular it inhibits 5HT-induced contractions in the isolated rat caudal artery and other blood vessels [2].

Receptor-binding studies in brain tissues [1] indicate that ketanserin specifically combines with a subpopulation of 5HT receptors indicated as '$5HT_2$ receptors' [1, 4, 7].

5HT induces a shape change in platelets from different animal species and brings about aggregation of human platelets [8, 9]. It is actively taken up by platelets which release it in response to various stimuli [10]. It has been observed [11] that the stimulatory effect of 5HT on platelet shape change and aggregation is unrelated to its active transport and that at least two separate 'receptors' for 5HT are present on rat [12] and human platelets [13, 14].

More recently the presence of functional $5HT_2$ receptors on human platelets different from those involved in its uptake was postulated by De Clerck *et al.* [15]. These authors have shown that ketanserin inhibits 5HT-induced and amplified platelet aggregation in human platelets ($IC_{50} = 1.66 \times 10^{-8}$ M) but does not affect its active uptake at concentrations below 5×10^{-6} M.

In the present study we compared the inhibitory effects of ketanserin on 5HT-induced shape change and 5HT uptake in human and rat platelets. The relative potency of ketanserin against the two platelet functions examined was compared to methysergide, a potent antagonist of 5HT-induced platelet shape change [12, 14] and chlorimipramine, a potent inhibitor of 5HT uptake by platelets [12, 14].

Materials and methods

Blood was collected from healthy donors of both sexes (20–30 years old) and from 250–300 g CD-COBS male rats (Charles River, Calco, Italy), on 3.8 and 3.1% trisodium citrate (ratio of anticoagulant/final sample volume 1:10) respectively, and processed as previously described [16] to obtain platelet-rich (PRP) and platelet-poor plasma (PPP).

Table 1. Apparent K_m values (μM) for 5HT-induced platelet shape change and platelet 5HT uptake in platelet-rich plasma from man or rat

	Shape change	Uptake
Human	0.30 ± 0.07	0.51 ± 0.01
Rat	0.31 ± 0.03	0.30 ± 0.04

Mean ± S.E. of 5 separate experiments.

Human PRP was used undiluted for uptake and shape change studies. Platelet count ranged between 350,000 and 450,000/μl. Rat PRP was diluted with autologous PPP to a final platelet count of about 400,000/μl for uptake studies and 200,000/μl for shape change studies.

Platelet uptake of [^{3}H]*5-hydroxytryptamine.* The method used was essentially that of Gordon and Olverman [17] as described by Bianchi *et al.* [18]. Briefly [^{3}H]5-hydroxytryptamine ([^{3}H]5HT) was added to 0.2 ml PRP preincubated at 37° for 2 min with saline or drug. The volumes of saline, drug solution and [^{3}H]5HT were selected so that the final sample volume was 0.24 ml. After 10 sec the reaction was stopped by adding 1 ml of cold 0.4% disodium EDTA in isotonic saline. The platelets were pelleted by centrifugation in an Eppendorf microcentrifuge (10,000 *g* for 40 sec), the supernatant was discarded and the tubes rinsed once with 1 ml of cold EDTA–saline.

The platelet pellets digested by 85% formic acid for 24 hr were resuspended in 5 ml of a scintillation mixture (AquaLuma-Supelchem) and counted for radioactivity in a liquid scintillation counter. Radioactivity trapped in the cell pellets or adhering to the tubes was measured by running parallel blank samples (samples cooled to 4°, [^{3}H]-5HT added after dilution with 1 ml of cold EDTA–saline and then processed as indicated above) [17].

Shape change. Samples of 0.25 ml PRP were preincubated at 37° for 1 min with 2.5–5 μl of either isotonic saline or different concentrations of test drug in an ELVI 840 aggregometer (Elvi Logos, Milan, Italy), under continuous stirring. The decrease in light transmission due to shape change [19], induced by addition of 2.5–5 μl of 5HT (at different concentrations) to the stirred PRP was continuously monitored by a pen recorder set at 0.1V full scale to ensure sensitivity 10 times that usually employed for aggregation studies [19]. Maximal decrease in light transmission was taken as a measure of platelet shape change [19].

Both uptake and shape change show a typical saturable dose–response curve and study conditions (duration of reaction, platelet, substrate and inhibitor concentrations) were selected to permit kinetic analysis of the data (Lampugnani *et al.*, manuscript in preparation). The ability of 5-HT to induce the two platelet responses, uptake and shape change, was expressed as K_m (apparent) values (Table I). K_m values were obtained by Lineweaver–Burk transformation [20] of the data. K_i (apparent) values were obtained by Dixon analysis [21] using at least five inhibitor concentrations and two substrate concentrations, one in the range of the K_m value and the other one at which the process was close to saturation. In all instances inhibition appeared to be competitive.

Materials. 5-Hydroxy[G-^{3}H]tryptamine creatinine sulphate (sp. act. 500 mCi/mmole, radiochemical purity 98%) was obtained from the Radiochemical Centre (Amersham, U.K.); 5-hydroxytryptamine creatinine sulphate from Fluka AG (Buchs, Switzerland); ketanserin (R49945, the tartrate salt of R41468) from Janssen Pharmaceutica (Beerse, Belgium); methysergide hydrogen maleate from Sandoz Ltd. (Basel, Switzerland) and chlorimipramine-HCl from Ciba-Geigy (Origgio, Italy) were dissolved and diluted in redistilled water.

Results and discussion

The comparative effects of ketanserin, methysergide and chlorimipramine on 5HT-induced shape change and 5HT uptake in human and rat platelets are summarized in Table 2. Ketanserin and methysergide both appeared to be much more effective as inhibitors of shape change than of 5HT uptake, whereas the opposite was true for chlorimipramine. Ketanserin was more effective on human than on rat platelet shape change, methysergide vice versa. In all instances, however, the K_i values ranged between 0.02 and 0.1 μM.

Ketanserin was 5–10 times more active than methysergide on 5HT uptake by human and rat platelets. It follows therefore that the specificity of both compounds as inhibitors of 5HT-induced shape change (as indicated by the ratio of K_i uptake over K_i shape change) was lower for ketanserin than for methysergide. Nevertheless, the ratio of the K_i of ketanserin against 5HT uptake versus 5HT-induced shape change was 314 for rat and 3100 for human platelets (Table 2). Interestingly enough, ketanserin was more specific on human platelets whereas methysergide was more specific on rat platelets.

Neither compound (up to 300 μM concentration) inhibited ADP-induced shape change. As expected, chlorimipramine inhibited 5HT uptake more than 5HT-induced shape change. It was more active on human than on rat platelets, in agreement with previous observations [16]. It was also more specific in human platelets.

The relative lack of effect of methysergide and ketanserin on 5HT uptake supports the suggestion [12–14] that a specific receptor for 5HT exists in human and rat platelets, distinct from the uptake site and responsible for starting the platelet shape change. Whether such a receptor behaves the same way as the brain $5HT_2$ receptors to which ketanserin specifically combines remains to be established.

Inhibition of the shape change by a high concentration of chlorimipramine supports previous data [12, 14] and indicates that some uptake inhibitors at high concentrations act as 5HT antagonists [22].

In summary, the present findings are consistent with the view that stimulation of platelet function by 5HT and its pharmacological inhibition can proceed independently from any interaction with the monoamine uptake. site. Ketanserin, a specific $5HT_2$ receptor antagonist on rat fron-

Table 2. Apparent K_i values (μM) for ketanserin, methysergide and chlorimipramine on 5HT-induced shape change and [^{3}H]5HT uptake in platelet-rich plasma from rat or man

	Rat			Human		
	Shape change	Uptake	Ratio*	Shape change	Uptake	Ratio*
Ketanserin	0.102 ± 0.013	32 ± 1.7	314	0.020 ± 0.003	62 ± 7.20	3100
Methysergide	0.017 ± 0.003	310 ± 25	18,235	0.046 ± 0.012	394 ± 14.0	8565
Chlorimipramine	0.631 ± 0.1	0.033 ± 0.004	19	0.700 ± 0.20	0.006 ± 0.0009	116

Mean ± S.E. of 5 separate experiments.

* Ratio: for ketanserin and methysergide, the K_i on uptake over the K_i on shape change; for chlorimipramine, the K_i on shape change over the K_i on uptake.

tal cortex binding sites [1], is markedly more potent as an inhibitor of 5HT-induced shape change than of 5HT uptake. However, the compound is less specific for 5HT-induced shape change than for active uptake when compared with methysergide which in brain receptor binding studies acts on $5HT_1$ and $5HT_2$ receptors [1].

In agreement with earlier observations [23], therefore, the present study does not provide unequivocal evidence of an analogy between the types of brain receptor for 5HT and those involved in functional platelet changes induced by 5HT.

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REFERENCES

1. J. E. Leysen, F. Awouters, L. Kennis, P. M. Laduron, J. Vandenberk and P. A. J. Janssen, *Life Sci.* **28**, 1015 (1981).
2. J. M. Van Nueten, P. A. J. Janssen, J. Van Beek, R. Xhonneux, T. J. Verbeuren and P. M. Vanhoutte, *J. Pharmac. exp. Ther.* **218**, 217 (1981).
3. J. C. Demoulin, M. Bertholet, D. Soumagne, J. L. David and H. E. Kulbertus, *Lancet* **i**, 1186 (1981).
4. J. E. Leysen, C. J. E. Niemegeers, J. P. Tollenaere and P. M. Laduron, *Nature, Lond.* **272**, 168 (1978).
5. F. Awouters, J. E. Leysen, F. De Clerck and J. M. Van Nueten, in *5-Hydroxytryptamine in Peripheral Reactions* (Eds. F. De Clerck and P. M. Vanhoutte). Raven Press, New York (in press).
6. J. Symoens, in *5-Hydroxytryptamine in Peripheral Reactions* (Eds. F. De Clerck and P. M. Vanhoutte). Raven Press, New York (in press).
7. S. J. Peroutka, R. M. Lebovitz and S. H. Snyder, *Science* **212**, 827 (1981).
8. W. J. Dodds, in *Platelets: A Multidisciplinary Approach* (Eds. G. de Gaetano and S. Garattini), p. 45. Raven Press, New York (1978).
9. F. De Clerck and J. L. David, *J. cardiovasc. Pharmac.* **3**, 1388 (1981).
10. G. de Gaetano, in *Platelets: A Multidisciplinary Approach* (Eds. G. de Gaetano and S. Garattini), p. 373. Raven Press, New York (1978).
11. G. V. R. Born, K. Juengjaroen and F. Michal, *Br. J. Pharmac.* **44**, 117 (1972).
12. A. H. Drummond and J. L. Gordon, *Biochem. J.* **150**, 129 (1975).
13. J. R. Peters and D. G. Grahame-Smith, *Eur. J. Pharmac.* **68**, 243 (1980).
14. A. Laubscher and A. Pletscher, *Life Sci.* **24**, 1833 (1979).
15. F. De Clerck, J. L. David and P. A. J. Janssen, *Agents Acts* (in press).
16. M. Wielosz, M. Salmona, G. de Gaetano and S. Garattini, *Naunyn-Schmiedeberg's Archs. Pharmac.* **296**, 59 (1976).
17. J. L. Gordon and H. J. Olverman, *Br. J. Pharmac.* **62**, 219 (1978).
18. L. Bianchi, L. Stella, G. Dagnino, G. de Gaetano and E. C. Rossi, *Biochem. Pharmac.* **30**, 709 (1981).
19. G. V. R. Born, *J. Physiol.* **209**, 487 (1970).
20. H. Lineweaver and D. Burk, *J. Am. chem. Soc.* **56**, 658 (1934).
21. M. Dixon, *Biochem. J.* **55**, 170 (1953).
22. R. Domenjoz and W. Theobald, *Archs int. Pharmacodyn. Ther.* **120**, 450 (1959).
23. M. Graf and A. Pletscher, *Br. J. Pharmac.* **65**, 601 (1979).

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Inhibition and enhancement of mixed-function oxidases by nitrogen heterocycles

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Mixed-function oxidases (MFO) are inhibited, both *in vitro* and *in vivo*, by several classes of organic compounds. Many nitrogen heterocycles, including imidazoles [1–3], benzimidazoles [4], and pyrroles [5], are effective inhibitors of MFO activities.

Aromatic hydroxylations, particularly the *para*-hydroxylation of aniline, are capable of being enhanced by a range of xenobiotic molecules. Aniline *p*-hydroxylase activity is enhanced in the presence of ethyl isocyanide [6], acetone and butanone [7], 2,2′-dipyridyl [8], and acetophenone [9]. Even though several ketones and pyridine-containing compounds enhance microsomal MFO activity, no generalisations regarding the structural requirements for enhancement have been made.

The present study was undertaken to examine further if any particular classes of compounds could be categorised as enhancers of aniline *p*-hydroxylation and to examine whether any relationships exist between the inhibition of aminopyrine *N*-demethylase (APDM) and the enhancement of aniline *p*-hydroxylase (APH).

5(6)-Benzoylbenzimidazole (I) was synthesised by the reaction of 4-benzoyl-*o*-phenylenediamine with formic acid. The yield was 20%, and the compound melted at 143–145° (microanalysis: $C_{14}H_{10}N_2O$; calc. C 75.7%, H 4.5%, N 12.6%; found C 75.5%, H 4.7%, N 12.5%). 6-Ethoxy-2-methylbenzoxazole (III) was synthesised from *m*-ethoxyphenol after nitration at 25–35° in acetic acid/nitric acid (1:1) and reduction in a Parr hydrogenation apparatus with Raney nickel and acetic anhydride/acetic acid solvent. Cyclisation was effected in 10% acetic anhy-