The combination of equations 3 and 5 leads to equation 6 which enables the binding affinity for bovine serum albumin of all 20 compounds to be modelled.

$$\log k = -0{\cdot}15\,\pi^2 + 0{\cdot}25\,\pi - 0{\cdot}19\,\sigma + 6{\cdot}55 \quad (6)$$
$$(\pm 0{\cdot}1) \quad (\pm 0{\cdot}12)\ (\pm 0{\cdot}07)$$

Compounds 1–20, R = 0·94, F = 62, S = 0·09.

To conclude, it has been shown that the binding of *N*-phenylanthranilic acids to bovine serum albumin is governed mainly by hydrophobic forces, probably by van der Waals' interactions between the phenyl ring and a hydrophobic area on the protein. The relationship is probably a parabolic one and addition of substituents with too great a hydrophobicity will lead to a decrease in binding affinity. Binding can also be modified by the presence of strong electron withdrawing substituents such as NO_2 which will reduce the availability of π electrons in the phenyl ring for van der Waals' interactions.

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Structural requirements for competitive α-adrenoceptor occupancy by cyclic and opened analogues of WB 4101

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Benzodioxanes represents one of the oldest class of reversible antagonists of the α-adrenoceptor (Melchiorre & Belleau 1981). Few of them were introduced in therapy but they were eventually discarded because of their severe adverse effects. All the compounds of this series have a benzodioxane nucleus as the main structural feature. Structure-activity relationships studies have shown that small structural manipulations may produce a pronounced difference in pharmacological activity. The most active of the series, WB 4101, was reported to be a very potent and selective postsynaptic α_1-blocker with an unusually high pA_2 value (Mottram & Kapur 1975; Kapur & Mottram 1978; Kapur et al 1978, 1979a, 1979b). It is being widely used for the characterization of the α_1-receptor through binding studies either in the brain or periphery (Kapur et al 1979b; Raisman et al 1979; Atlas & Adler 1981). Both benzodioxane and 2,6-dimethoxyphenoxyethyl moieties were reported to be essential for activity (Kapur et al 1978). In fact, the substitution of oxygen at position 4 with a methylene gives rise to a significant decrease in activity. On the other hand, changing the ethoxy moiety, or removal of one or both methoxy groups, greatly reduces the potency compared with WB 4101. One aspect of structure-activity relationships which appear to have escaped attention until now concerns the possibility that the target site of WB 4101 may have multiple identical or quasi-indentical subsites.

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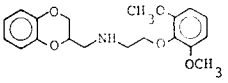
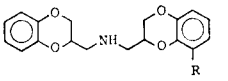
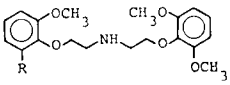
In fact, a close inspection of the chemical structure of WB 4101 reveals that the benzodioxane nucleus may be regarded as the desmethoxy cyclic moiety of the 2,6-dimethoxyphenoxymethyl group and vice versa. Thus a symmetric or quasi-symmetric molecule can be designed which might promote a better fit with the adrenergic α-receptor. Dibozane, another α-antagonist of benzodioxanes class, is actually a symmetric molecule. However, it incorporates two basic nitrogens and hence the distance between the two benzodioxane nuclei is longer than that of WB 4101 (Melchiorre & Belleau 1981). Nevertheless, dibozane displays high α-blocking activity which may suggest the interaction with symmetric sites. This reasoning may be justified if one considers that the α-receptor surface incorporates symmetrically at least four anionic sites linearly arranged near a buried target thiol (Melchiorre 1981; Melchiorre & Belleau 1981). Thus, it is theoretically possible that the receptor surface, in addition to these anionic sites, might well incorporate other (aromatic) sites disposed symmetrically. With this goal in mind, we have evaluated some drugs structurally related to WB 4101.

Method

Vasa deferentia from male albino rats (175–200 g) were mounted individually in 10 ml organ baths containing Krebs bicarbonate buffer. The medium was maintained at 37 °C while being aerated with 95% O_2 – 5% CO_2.

Table 1. Antagonistic effects of WB 4101 and structurally related drugs on noradrenaline-induced contraction of isolated rat vas deferens after 5 min incubations.

Drug[a]		pA_2[b]	Slope
WB 4101		8·83 ± 0·02	0·94
I: R = H		6·38 ± 0·10	0·89
II: R = OCH_3		7·42 ± 0·09	1·01
III: R = H		8·21 ± 0·09	0·72[c]
IV: R = OCH_3		6·41 ± 0·07	0·81

[a] All drugs were tested as hydrochloride salts. [b] pA_2 values plus or minus standard error of estimate were established by using Schild plots. The log (DR-1) was calculated at four different concentrations and each concentration was tested at least 4–6 times. [c] Significantly different ($P < 0·01$) when compared with the mean value of II and WB 4101.

The loading tension was 0·40 g and the contraction were recorded by means of force transducers connected to a two channel Gemini 7070 poligraph. The antagonistic potencies of the drugs were evaluated against noradrenaline by measuring their pA_2 value (Arunlakshana & Schild 1959).

All drugs used (Table 1) were synthesized in our laboratory and their synthesis will be reported elsewhere. The drugs were: 2-[(2′,6′-dimethoxyphenoxyethylaminomethyl]-1,4-benzodioxane (WB 4101); bis(1,4-benzodioxan-2-ylmethyl)amine (I); (8-methoxy-1,4-benzodioxan-2-ylmethyl)(1,4-benzodioxan-2-ylmethyl)amine (II); [2-(2′,6′-dimethoxy)phenoxyethyl] [2-(2′methoxy)phenoxyethyl]amine (III) and bis[2-(2′,6′-dimethoxy)phenoxyethyl]amine (IV). The drugs were dissolved immediately before each experiment with 90% ethanol.

Results and discussion

The results reported in Table 1 clearly indicate that the drugs all display high postsynaptic α_1-receptor blocking activity. Furthermore, the fact that the slope is close to unity suggests that the antagonism is competitive. Doubling the benzodioxane nucleus or 2,6-dimethoxyphenoxyethyl moiety affords significantly weaker antagonists (I and IV) compared with WB 4101. This indicates that the two moieties linked to the nitrogen do not bind at two identical areas on the receptor surface. Nevertheless, the high activity displayed by the cyclic and opened analogues of WB 4101 (II and III) might suggest that the binding site(s) incorporates at least two very similar areas with quasi-identical structural requirements. In addition, the opened analogue III with a pA_2 of 8·21 clearly shows that the benzodioxane nucleus may not be essential to activity. The high activity of III can hardly be ascribed to an unusual reductive cyclizing process to WB 4101 in the physiological solution before receptor interaction. However, the significant deviation ($P < 0·01$) from unity in the slope of III might indicate a drug-receptor interaction mechanism not truly competitive which may be indeed the result of binding with different receptor sites. In fact, it was shown that benzodioxanes interact at least with two distinguishable sites (Avner & Triggle 1974). Furthermore, it was reported very recently that WB 4101 binds in addition to the α-receptor to calcium channels (Atlas & Adler 1981). Whether these findings apply to I–IV remains to be ascertained. However, rigid molecules have higher stereochemical requirements and may have a higher chance of selectivity in action than flexible molecules. In this regard, the cyclic analogue II might result in an useful labelling agent in the characterization of the adrenergic α-receptor.

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