Further studies on receptor interaction for the α-antagonist WB 4101

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WB 4101, a member of the series of benzodioxanes previously described by Fenton et al (1965), has been shown to produce profound postsynaptic α-adrenoceptor antagonism at both peripheral (Mottram & Kapur 1975) and central (Greenberg et al 1976) α-adrenoceptors. The results of a recent study (Kapur et al 1978) suggest that the postsynaptic α-adrenoceptor antagonism exhibited by WB 4101 depends not only on the benzodioxan moiety but that incorporated into the receptor is at least one other subsite for aromatic interaction at a specific distance from the subsite for nitrogen interaction and that this tertiary interaction may account for the very high potency of WB 4101. The results also suggested that the 2,6-dimethoxy substituents play an important role in the drug-receptor interaction since re-positioning of the methoxy groups around the phenyl ring, or their removal, greatly reduces α-antagonism.

The present study was undertaken to study a further series of compounds related to the potent α-blocker WB 4101 with particular interest in the effects of alteration of the 1,4-benzodioxan moiety on post-synaptic α-blockade.

Vasa deferentia from male Wistar rats (200–300g) were suspended in 10 ml organ baths and bathed in Tyrode solution (composition, g litre^{-1}, NaCl 8·0, KCl, 0·2, $MgCl_2.6H_2O$, 0·2, $CaCl_2$, 0·2, $NaH_2PO_4.H_2O$, 0·05, $NaHCO_3$, 1·0 and glucose, 1·0) maintained at 37 °C and aerated with a mixture of 5% CO_2 in oxygen. Isometric contractions were recorded with Devices 2oz strain gauge transducers and two channel recorders.

The antagonistic potencies of the compounds were evaluated against noradrenaline by measuring their pA_2 values (Schild 1947), and results are presented in Table 1. Three major substitutions were made for the 1,4-benzodioxan group of WB 4101 these being the groups chroman (WB 4346), 2,3-dihydrobenzofuran (WB 4174) and benzofuran (WB 4249). All three substituents produced a substantial fall in the postsynaptic α-blocking activity of the compounds, compared with WB 4101. The reduction in activity with these three analogues may be directly related to the relative degree of rigidity inherent in these ring structures and the effect this rigidity has on the relative positioning of the aromatic ring to the nitrogen atom, the primary site for drug receptor interaction on the postsynaptic α-adrenoceptor. These results therefore indicate that an aromatic subsite for the benzodioxan group indeed exists within the receptor, and at a specific distance from the primary nucleophilic site.

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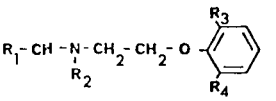
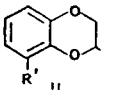
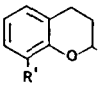
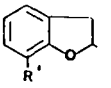
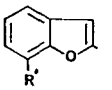
Table 1. pA_2 values ± s.e.m. of compounds related to the benzodioxan WB 4101 (2-(N-[2,6-dimethoxyphenoxyethyl]aminomethyl-1,4-benzodioxane).



R_2 = H except for WB4116 when R_2 = Me

Compound	R_1	R_3	R_4	pA value
WB4101 R'= H		OMe	OMe	9.80 ± 0.04(6)
WB4116 R'= H	"	"	"	5.49 ± 0.07(4)
WB4267 R'= Me	"	"	"	7.5 ± 0.08(4)
WB4346 R'= H		"	"	7.35 ± 0.07(5)
WB4404 R'= H	"	"	H	6.77 ± 0.05(5)
WB4405 R'= H	"	H	H	5.39 ± 0.05(5)
WB4400 R'= Me	"	OMe	OMe	7.19 ± 0.02(5)
WB4174 R'= H		"	"	6.26 ± 0.06(5)
WB4172 R'= H	"	"	H	6.02 ± 0.02(4)
WB4265 R'= H	"	H	H	4.17 ± 0.07(4)
WB4204 R'= Me	"	OMe	OMe	6.32 ± 0.06(5)
WB4249 R'= H		OMe	OMe	4.88 ± 0.07(5)

In a previous report (Kapur et al 1978) it was noted that removal of the methoxy groups from the dimethoxyphenoxyethyl moiety of WB 4101 produced a graded decrease in α-blocking activity, suggesting an important role for these methoxy groups in the receptor interaction for WB 4101. This conclusion is ratified in the present series of experiments in that substitution of the methoxy groups from the dimethoxyphenoxyethyl moieties of WB 4346 and WB 4174 again produced a graded decrease in α-blocking activity.

The effect of adding a methyl group to the benzene ring of the analogues under investigation was also studied. A negligible effect on α-blockade was noted when the methyl group was added to the 2,3-dihydrobenzofuran (WB 4204) or 2,3-dihydrobenzo-gamma-pyran (WB 4400) moieties, whilst over a hundred-fold decrease in activity was recorded when a methyl group was added to the 1,4-benzodioxan moiety (WB 4267). From these results it would appear that the benzene ring in WB 4204 and WB 4400 plays little part in the drug-receptor interaction probably due to the previously described rigidity and altered conformation of these molecules, and that substitution of a methyl group into the 1,4-benzodioxan causes shift in the alignment of the benzene ring and therefore intereferes with its interaction with its sub-site within the receptor. This observation is confirmed by the closeness in pA_2 values for WB 4346 and WB 4267 which closely resemble WB 4101 but where the removal of an oxygen and addition of a methyl group respectively has essentially produced the same effect, in that the benzene ring is unable to interact as effectively with the receptor.

Finally the effect of methyl substitution on the amino group was investigated and, expectedly, a dramatic fall in α-blocking activity was observed reflecting the importance of the secondary amine in drug-receptor interaction on the α-adrenoreceptor.

In conclusion the results of the present study provide further evidence for the unique α-receptor blocking activity of WB 4101 and show that substitution of any part of the molecule produces a detrimental effect on drug receptor interaction. In particular the aromatic group within the benzodioxan moiety can clearly be seen to play an important role in the interaction with the α-adrenoceptor and that the subsite within the receptor with which it interacts lies within a specific sphere of influence to the primary nucleophilic subsite within the receptor. Together with previous results (Kapur et al 1978) these findings support the view that the α-adrenoceptor has at least two aromatic subsites, at specific distances from the primary site of binding, through which α-adrenoceptor antagonists may interact, and that the ability of WB4101 to bind to both aromatic subsites accounts for its exceptional potency as an α-adrenoceptor blocker.

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The effect of 2-2′-pyridylisatogen tosylate on the increase in capillary permeability produced by ATP

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Antagonism by 2-2′-pyridylisatogen tosylate (PIT) of the inhibitory action of ATP on guinea-pig isolated taenia caeci was first described by Hooper et al (1974) and Spedding et al (1975). ATP also acts on rat mast cells to produce degranulation and release of amines (Kiernan 1972) which cause vasodilatation and increase in capillary permeability, and it has been implicated in the phenomenon of antidromic vasodilatation (Holton 1959). Since an ATP antagonist had not previously been described, experiments were performed to determine whether PIT antagonized the action of ATP in increasing capillary permeability in the hope that it might be a useful drug for investigation of the possible role of ATP in antidromic vasodilatation and neurogenic oedema.

Dye leakage responses to ATP and other irritants injected into the abdominal skin of rats given Evans blue intravenously were quantified using the method described by Harada et al (1971). Interactions of PIT with the irritants tested were assessed by the method described by Chahl (1977) for testing interaction of substance P with mediators of inflammation. The amount of dye extracted from the skin by a mixture of acetone and sodium sulphate (0·5%) was measured spectrophotometrically and expressed as absorbance.

In several rats the effect of PIT injected intracutaneously on the response to ATP was measured. In these experiments injections of ATP were given into the abdominal skin in the presence and absence of various concentrations of PIT and the results analysed by paired *t*-tests. The results are shown in Fig. 1a. PIT inhibited responses to ATP (2×10^{-7} mol) with significant inhibition occurring with PIT (5×10^{-9} mol) ($0{\cdot}05 > P > 0{\cdot}01$) and PIT ($5 \times 10^{-11}$ mol) ($0{\cdot}01 > P > 0{\cdot}001$) but not with PIT ($5 \times 10^{-8}$ mol) or (5×10^{-10} mol). The degree of inhibition varied from one rat to another and was not marked. The highest dose of PIT (5×10^{-8} mol) produced dye leakage of a similar magnitude to the responses to ATP (2·5 to 10×10^{-8} mol) (Fig. 1a). Lower doses of ATP were not significantly blocked by PIT (5×10^{-9} mol) (Fig. 1b). In similar experiments PIT (5×10^{-9} mol) was found to produce significant potentiation of responses to 5-hydroxytryptamine (5-HT) ($2{\cdot}5 \times 10^{-10}$ mol), histamine ($2{\cdot}5 \times 10^{-8}$ mol), substance P (Peptide Institute, Osaka, Japan) (5×10^{-10}