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}

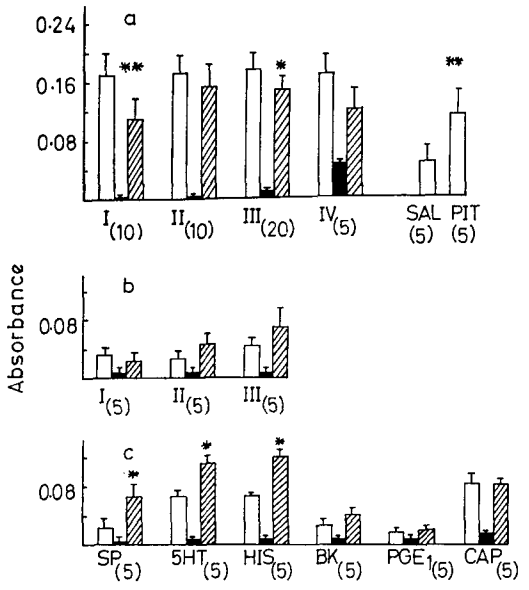

FIG. 1. Effect of 2-2′-pyridylisatogen tosylate (PIT) on the dye leakage responses to ATP and other inflammatory agents.
Histograms represent mean values expressed as absorbance and vertical bars are standard errors. Blank histograms are mean responses to ATP or other inflammatory agents alone; solid histograms represent responses to PIT alone, and cross-hatched histograms are responses to combinations of PIT with ATP or other inflammatory agents. Values in parentheses are the number of individual responses used for calculation of means. Asterisks indicate significant effects of PIT.
* $0{\cdot}05 > P > 0{\cdot}01$; ** $0{\cdot}01 > P > 0{\cdot}001$

(a) Shows responses to ATP (2×10^{-7} mol) in presence and absence of 4 doses of PIT (5×10^{-11} mol) (I); (5×10^{-10} mol) (II); (5×10^{-9} mol) (III); (5×10^{-8} mol) (IV). Note reduced responses to ATP in presence of PIT. PIT (5×10^{-8} mol) alone produced a response. At the right of this figure are responses to ATP (2×10^{-7} mol) obtained on skin sites pretreated 30 min before with saline (SAL) and with PIT (5×10^{-9} mol) (see text).

(b) Shows the effect of PIT (5×10^{-9} mol) on responses to lower doses of ATP ($2{\cdot}5 \times 10^{-8}$ mol) (I); (5×10^{-8} mol) (II); and (1×10^{-7} mol) (III).

(c) Shows responses in the presence and absence of PIT (5×10^{-9} mol) to substance P (SP) (5×10^{-10} mol); 5HT ($2{\cdot}5 \times 10^{-10}$ mol); histamine (HIS) ($2{\cdot}5 \times 10^{-8}$ mol); bradykinin (BK) (5×10^{-10} mol); prostaglandin E_1 (PGE_1) (5×10^{-10} mol); and capsaicin (CAP) (5×10^{-8} mol). Note enhanced responses to SP, 5HT and HIS in presence of PIT.

mol) (all $0{\cdot}05 > P > 0{\cdot}01$) but not of responses to bradykinin (5×10^{-10} mol), prostaglandin E_1 (5×10^{-10} mol) or the neurogenic oedema producing agent, capsaicin (5×10^{-8} mol) (Arvier et al 1977) (Fig. 1c). Increasing the time of contact of the abdominal skin sites with PIT by pretreatment of sites with intracutaneous injections of PIT (5×10^{-9} mol) 30 min before injection of ATP caused significant enhancement ($0{\cdot}01 > P > 0{\cdot}001$) of the response to ATP compared with the reduced responses at sites pretreated with saline (Fig. 1a).

Intravenous injection of PIT (4×10^{-7} mol), either 30 min before, or immediately before intracutaneous injections, produced no significant change in the responses to ATP (2×10^{-7} mol), 5-HT ($2{\cdot}5 \times 10^{-10}$ mol), histamine ($2{\cdot}5 \times 10^{-8}$ mol), substance P (5×10^{-10} mol) or capsaicin (5×10^{-8} mol) as assessed by Student *t*-test (Table 1). Higher doses of PIT could not be investigated because of its limited solubility.

Table 1. Effect of intravenous PIT (4×10^{-7} mol) on dye leakage responses to inflammatory agents[1].

Control	Response[2] immediately after PIT	Control	Response[3] 30 min after PIT
ATP (2×10^{-7} mol)			
0·317 ±0·099	0·224 ±0·013	0·121 ±0·015	0·237 ±0·056
Substance P (5×10^{-10} mol)			
0·045 ±0·012	0·039 ±0·008	0·158 ±0·030	0·118 ±0·019
5-HT ($2{\cdot}5 \times 10^{-10}$ mol)			
0·076 ±0·010	0·116 ±0·016	0·073 ±0·015	0·102 ±0·010
Histamine ($2{\cdot}5 \times 10^{-8}$ mol)			
0·073 ±0·028	0·104 ±0·023	0·101 ±0·023	0·113 ±0·026
Capsaicin (5×10^{-8} mol)			
0·063 ±0·017	0·076 ±0·011	0·093 ±0·018	0·080 ±0·017

[1] Values shown are mean absorbances ± s.e. from 5 animals.
[2] Values in this column were from animals given PIT i.v. immediately before intracutaneous injection of inflammatory agents.
[3] Values in this column were from animals given PIT i.v. 30 min before intracutaneous injection of inflammatory agents.
No significant changes occurred with either PIT treatment.

From these experiments it was concluded that, although PIT did show some antagonism toward this response to ATP possibly by inhibiting mast cell receptors, in high doses it also produced dye leakage and in lower doses enhancement of responses to several substances including 5-HT and histamine, which are released from the mast cells. This might account for the variability of the antagonistic action exhibited by PIT against ATP. For this reason it would seem that PIT is not a satisfactory antagonist for ATP in this system. Spedding & Weetman (1978) have also recently drawn attention to the problems associated with the use of PIT as an ATP antagonist because of its many actions.

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Patency of chronic jugular cannulae with systematically varied outer diameter and length

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Chronic intrajugular cannulation has a variety of applications in biological investigations of the rat since it allows for repeated administration of substances to freely moving, and relatively stressfree subjects (Davis & Campbell 1974). There are a number of cannulation procedures differing in the dimensions of the tubing inserted into the vein. The tubing, typically silicone because of its reduced tendency to cause tissue irritation (Stewart & Sanislow 1961), has its termination ranging from a point in the heart (Weeks 1972) to one a few cm from the heart (Davis & Campbell 1974), and its diameter varied between 0·064 cm (Weeks 1972) and 0·1 cm (Steffens 1969).

The problem is that none of the procedures are entirely problem-free. For example, we have found in preliminary work that cannulae constructed and implanted according to Davis & Campbell (1974) often failed within 1 week of surgery. Similarly, Terkel & Urback (1974) noted problems with Week's cannula, although the implication was that the patency lasted longer than in Davis and Campbells' procedure. However, except that in many reports Week's cannula has been used, there are almost no reasons for choosing one cannula over another. Thus, the question to be answered in the present experiment was whether any particular cannula has a higher probability of remaining patent.

Fifty-four adult male Wistar rats (Canadian Breeding Laboratories), housed individually in stainless steel cages, were divided into six equal groups. Under sodium pentobarbitone anaesthesia (60 mg kg^{-1}) each rat received one of six different cannulae, implanted into the right jugular vein. The cannulae varied over two lengths and three diameters. They were constructed by swelling silicone tubing on to the end of 15 cm of polyethylene tubing. A 10 cm length of cotton suture was secured with a knot over the joint of the connection and the silicone tubing portion of the cannula was cut to a length of 25 mm according to Davis & Campbell (1974), or 40 mm to rest just inside the right atrium as recommended by Weeks (1972). The cut end of the silicone tubing was then plugged with cement (Dow Corning Silastic No 891) and after curing, pin holes (1 mm^{-2}) were punched over the final 0·75 cm. The three diameters of the cannulae, 0·064, 0·094, and 0·119 cm, were constructed of Silastic tubing No 602–101 and Clay Adams Intramedic PE 10, Silastic No 602–135 and PE 50, and Silastic No 602–151 and PE 60, respectively. The tubing diameters were the three sizes of silicone tubing available to us which were capable of easily fitting into the jugular vein.

Surgery was similar to the methods of Weeks (1972) and of Davis & Campbell (1974). The right jugular vein was exposed through a 3 cm skin incision following the separation of the fat and connective tissue. The cannula was inserted into the blood vessel about 1 mm rostral to the cephalic vein and was gently pushed until the aforementioned knot came in contact with the opening into the vein. The cannula was secured, once to the pectoral muscle and twice to one of the adjacent neck muscles. The cannula was then passed subcutaneously to an exit site 2 cm behind the ears where the tube was sutured once to the outside of a stab wound. Each rat received no systematic post-

Table 1. Proportion of rats with patent cannulae in groups implanted with cannulae having one of three diameters and two lengths.

Cannula outer diameter (mm)	0·064	0·094	0·119
25 mm length	5/9	0/8	0/7
40 mm length	9/9	6/6	3/9

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