Short communications

Some actions of sympathomimetic amines and their antagonists on mouse superfused ileum

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Superfused segments of mouse ileum exhibited spontaneous movement which were inhibited by sympathomimetic amines. Isoprenaline and adrenaline were approximately equipotent and about 15 times more potent than either noradrenaline or phenylephrine. Supersensitivity developed to all these agents when mice had been 'sympathectomized' with NGF-antiserum or 6-hydroxydopamine and it was more pronounced with the latter treatment. Responses to the amines were reduced by phentolamine and propranolol, alone or in combination, with the exception of phenylephrine, the responses to which were simply shortened in duration after β-adrenoceptor blockade. No qualitative changes were evident in the effects of the blocking agents after 'sympathectomy'.

Administration of nerve growth factor antiserum (NGF-As) to new born mice or 6-hydroxydopamine (6-OHDA) to mice of any age will cause an impairment of sympathetic nervous system function. In a previous publication (Hughes, Kirk, Kneen & Large, 1973) we compared the 'sympathectomy' produced by these agents by assessing both the responsiveness to nerve stimulation and the sensitivity towards noradrenaline of various tissues taken from treated mice. Although supersensitivity of atria and vasa deferentia to noradrenaline was clearly shown, no such distinction was obvious in segments of ileum from 'sympathectomized' mice. The present communication describes a more detailed study of the effects of sympathomimetic amines and adrenoceptor blocking agents on superfused mouse ileum where the experiments were not complicated by electrical stimulation of the sympathetic nerves.

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Methods.—Male and female mice were used of Tuck No. 1 strain, 8 to 13 weeks old which had been inbred in our laboratories for 4 generations. Two litters were injected s.c. with NGF-As 0·05 ml given daily for 5 successive days during the first 8 days *post partum*, whilst three litters received instead 0·9% NaCl. Mice from the latter group treated with 6-OHDA had two separate injections of 50 mg/kg i.v., followed 8 days later by a single injection of 100 mg/kg and, 24 h after this third dose, they were killed, as were the other mice, by cervical dislocation.

Isolated segments of small intestine, about 1·5 cm long, were suspended in air inside a heated water jacket and superfused with McEwen's (1956) solution (mM: NaCl 130·0, KCl 5·6, $CaCl_2$ 2·1, NaH_2PO_4 1·2, $NaHCO_3$ 24·9, glucose 11·1 and sucrose 13·1) gassed with 5% carbon dioxide in oxygen at 32°C flowing by gravity at a rate of 0·5 ml/minute. Movements were recorded through an isotonic transducer (load 850 mg) and displayed on a Heathkit chart recorder after suitable amplification. Sympathomimetic amines ((−)-adrenaline hydrogen tartrate, (−)-noradrenaline bitartrate, (−)-phenylephrine hydrochloride and (±)-isoprenaline sulphate) were applied to the tissue through separate tubing at the same flow rate for 20 s periods (optimal contact time) on a 3 min cycle, during which time the drug-free solution was diverted from the tissue. Adrenoceptor blocking agents were superfused for 15 min prior to, and during the intervening periods of agonist responses.

In each experiment a dose-response curve to a particular agonist was first determined. Then a dose sufficient to produce about 50% maximal response was chosen and alternated with the remaining agonists which were applied in amounts sufficient to give responses similar in size; thus the effect of an antagonist on each drug could be assessed. The upper edge of the peristaltic record was used in measuring the responses which were expressed as percentages of the maximum attained in the dose-response determination. Amounts of sympathomimetic amines are given as ng of base to which the tissue was exposed during a 20 s period.

Results.—Superfused mouse ileum preparations possessed a high degree of tone and exhibited regular pendular movements.

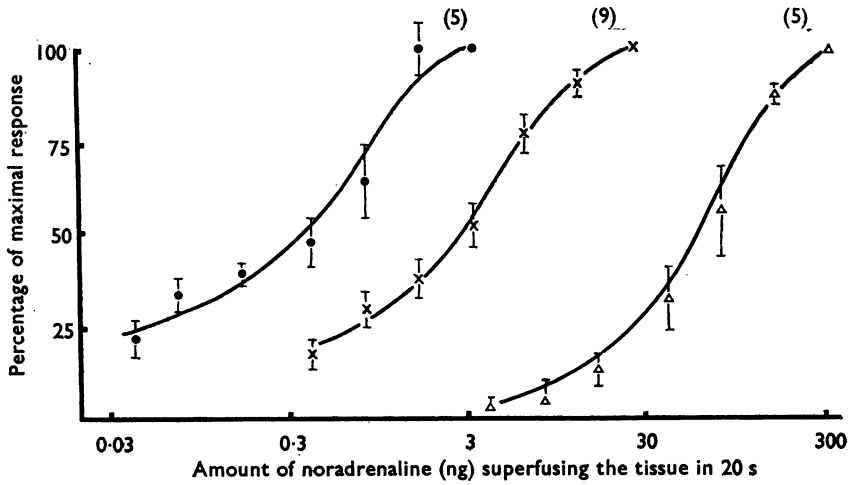

FIG. 1. Mouse superfused ileum: showing relationship between dose of noradrenaline (ng superfusing the ileum in 20 s) and response (percentage of maximum: mean ± S.E.M.) in the following groups, △——△ control, ×——×NGF-As and ●——● 6-OHDA. Figures in parentheses show numbers of experiments contributing to each point.

Low doses of the four sympathomimetic amines reduced the tone without affecting rhythm but as the amounts increased the pendular movements were eventually arrested and the ileum relaxed completely. Isoprenaline and adrenaline were about equipotent, amounts of 2 to 5 ng producing a 50% maximal response, whilst noradrenaline and phenylephrine were each about 15 times less potent than the former drugs. The response to isoprenaline was slow in onset, often reaching a maximum after the drug had been washed away, and pendular activity always began before the tissue had regained its initial tone. By contrast phenylephrine caused a rapidly developing relaxation followed by a similarly rapid recovery which, with the lower doses, often began whilst the drug was present. Occasionally an overshoot occurred similar to that seen in rabbit intestine by Bowman & Hall (1970). Responses to the other drugs were intermediate in character, noradrenaline more closely resembling isoprenaline and adrenaline similar to phenylephrine.

Dose-response curves to noradrenaline were also obtained in tissues from 'sympathectomized' mice (Figure 1). Supersensitivity to the amine was evident in both groups, more so after 6-OHDA treatment, and doses for 50% maximal responses were: control 53 ± 12·5 ng, NGF-As 3·2 ± 0·6 ng and 6-OHDA 0·42 ± 0·08 ng, with P values $<0{\cdot}001$ for intergroup comparisons (Student's t test). Although dose-response curves for the other drugs were not determined in these tissues, an indication of their effectiveness was gained by noting the amounts of each required for a 50% maximal response. Supersensitivity developed towards all the drugs, since about 1/20 and 1/100 of the usual dose was required in the NGF-As and 6-OHDA groups respectively to match the noradrenaline responses.

Effects of adrenoceptor blocking drugs

Concentrations of phentolamine mesylate and propranolol hydrochloride (1 μg/ml and 0·5 μg/ml respectively) were chosen which reduced adrenaline responses by about 50%. Somewhat surprisingly, noradrenaline was the drug least affected by phentolamine, followed by phenylephrine, then adrenaline and finally isoprenaline, the responses to which were reduced by about 80%. A similar order of inhibition occurred with propranolol except that responses to phenylephrine were this time reduced only in duration and not at all in amplitude. Qualitative changes were apparent in that after phentolamine the records obtained with all four amines were almost identical in shape to those produced initially by isoprenaline, and after propranolol they resembled the initial phenylephrine responses. A combination of the antagonists almost obliterated the responses to adrenaline and isoprenaline

and caused a greater inhibition of the other two amines than was anticipated from the foregoing results.

Similar effects were observed in the NGF-As and 6-OHDA groups, except that in the latter case phentolamine given alone completely abolished the responses to phenylephrine, adrenaline and isoprenaline and considerably reduced that to noradrenaline.

Discussion.—The reduction of mouse intestinal tone by sympathomimetic amines appears to involve two separate mechanisms which may be conveniently described in terms of α- or β-adrenoceptor activation. These observations are similar to those made on rabbit isolated intestine by Bowman & Hall (1970) in that α-adrenoceptor activation, such as is seen with phenylephrine applied alone or with the other amines in the presence of propranolol, typically caused relaxation which was rapid both in onset and recovery; by contrast these events were much longer in duration with β-adrenoceptor activation. It is of interest that a classification of adrenoceptors in rabbit intestine based on the rate of onset of drug action was used by van Rossum & Mujić (1965).

All four agonists can activate both types of receptor, since combined α- and β-adrenoceptor blockade caused a greater antagonism than either phentolamine or propranolol given alone, although only the duration and not the amplitude of phenylephrine-induced relaxation was affected by propranolol. Unlike rabbit intestine, responses of mouse ileum to isoprenaline were reduced by phentolamine, and although Hall (1972) described the development of tachyphylaxis to isoprenaline in rabbit intestine, such an explanation is unlikely here since not only did the reported tachyphylaxis take an hour or two to develop but also in our experiments combined α- and β-adrenoceptor blockade was more effective than either antagonist alone.

When judged solely on the basis of noradrenaline supersensitivity, 6-OHDA apparently produced a greater 'sympathectomy' than did NGF-As with our particular schedules of treatment. In another series of experiments (Hughes *et al.*, 1973) we showed that functional impairment of the sympathetic nerves was more pronounced in the heart than the intestine, with vas deferens unaffected by a similar course of NGF-As treatment, and that 6-OHDA was again more effective than NGF-As; all these differences may be simply related to the doses administered.

The dose-response curves to noradrenaline and the shifts after 'sympathectomy' were more clearly defined in the present experiments than in those previously reported by us. We can only suggest that this may have resulted from our confining the present experiments to an analysis of drug-induced responses whilst in the former experiments (Hughes *et al.*, 1973) frequency-response curves to electrical stimulation of periarterial nerves were always determined first. By our method of assessment, a similar degree of supersensitivity was observed to all four amines within the groups of treated mice. Sympathetic denervation supersensitivity is considered to be due in large measure to the absence of structures which normally remove chemical transmitter substances from the vicinity of receptors whose activation results in tissue responses. Iversen (1967) showed that rat perfused heart removed sympathomimetic amines to different degrees and that in descending order of uptake noradrenaline > adrenaline > isoprenaline > phenylephrine. We do not know the extent to which neuronal uptake mechanisms operate over the short periods of drug exposure in mouse superfused ileum, but unless uptake is very rapid and unless the intestinal nerves have different affinities from cardiac nerves for uptake, this explanation would not totally account for the observed supersensitivity. The results do not preclude the possibility of a 'spreading of receptors' after sympathectomy such as occurs with cholinoceptive sites after skeletal muscle denervation.

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