

An assessment of sympathetic function in isolated tissues from mice given nerve-growth-factor antiserum and 6-hydroxydopamine

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Summary

1. Experiments were done on isolated tissues from mice injected with 0.9% w/v NaCl solution (saline), 6-hydroxydopamine (6-OHDA), nerve-growth-factor antiserum (NGF-As) or a combination of these agents (NGF-As + 6-OHDA).
2. Fluorescence histochemistry of vasa deferentia showed clear differences between each of the treatments but no such distinction was possible in cardiac ventricle or intestine.
3. Compared with controls, the chronotropic responses of atria to field stimulation were reduced by all three treatments in the order NGF-As < 6-OHDA < NGF-As + 6-OHDA. Supersensitivity to noradrenaline occurred in atria from all treated groups.
4. In contrast, vasa deferentia from NGF-As-treated mice responded almost identically with controls to both field stimulation and to exogenous noradrenaline. Considerable functional impairment and noradrenaline supersensitivity were obvious in both the 6-OHDA and the NGF-As + 6-OHDA groups but no distinction could be made between them.
5. The relaxation of superfused ileum following nerve stimulation was reduced in the NGF-As group, whilst preparations from the two groups given 6-OHDA usually gave a motor response which was blocked by atropine.
6. None of the treatments employed in these experiments caused complete sympathectomy although 6-OHDA alone or in combination with NGF-As produced a more pronounced effect than NGF-As alone and the relative ineffectiveness of the latter is discussed. The implications of the motor response after 6-OHDA in ileum when the nerves were stimulated is considered in the light of the cholinergic link hypothesis.

Introduction

The production of a *total* 'sympathectomy' in experimental animals would be of great value in a variety of studies ranging from investigations of adrenergic nerve function to the genesis of hypertension. Axotomy of sympathetic trunks can be carried out but the effects produced are limited to the organs innervated by the sectioned axons, the technique is conditional on the presence of accessible post-ganglionic nerves and regeneration of some fibres frequently occurs. In addition, cholinergic nerves which may run in the sympathetic trunks will also be destroyed. Although many agents are capable of selectively inhibiting the function of adrenergic

nerves such inhibition is rarely associated with destruction of the nerve fibres. Two agents capable of destroying adrenergic nerves are nerve-growth-factor antiserum (NGF-As) (Levi-Montalcini & Booker, 1960; Zaimis, 1972) and 6-hydroxydopamine (6-OHDA) (Furness, Campbell, Gillard, Malmfors, Cobb & Burnstock, 1970; Malmfors & Thoenen, 1971). With the former agent, the effects are dependent on the state of development of the animal and its species. It is also possible that cholinergic sympathetic nerves will be destroyed. In the present paper, the effects of these two agents, either alone or in combination, have been investigated in the mouse by the measurement of responses of isolated atria, vas deferens and intestine to electrical stimulation of nerves, and to application of noradrenaline, and also by the use of a fluorescence histochemical technique.

Methods

Adult male mice, 10 to 13 weeks old (weight 29 to 40 g) of Tuck No. 1 strain were used. They had been inbred for 5 generations in our laboratories and all the mice given NGF-As were the first filial generation of parents which had been similarly treated at birth. On each of five days during the first eight days *post-partum*, mice were injected s.c. with 0.05 ml NGF-As and at the time of weaning males and females were separated. Other litters, born to parents treated with 0.9% w/v NaCl solution (saline) were injected s.c. with 0.05 ml saline over a similar period of time. Altogether, 18 mice were assigned to 4 experimental groups as shown in Table 1. At the appropriate time before being killed the mice were injected i.v. with either saline or 6-OHDA (made up freshly in saline which had been purged of dissolved O₂ by bubbling with N₂ and which contained 0.2% ascorbic acid). The drug was given in doses of 50 mg/kg twice, with 6 h between each injection on the 8th day prior to the experiment followed by a single injection of 100 mg/kg 24 h before being killed. The mice were used in a prearranged order based on a random block design and only one of us was aware of the group to which a particular mouse belonged.

TABLE 1. *Schedule of treatment and numbers of mice assigned to each group*

Group	Neonatal treatment	I.v. injections prior to experiment	
		Saline	6-OHDA
Control	Saline	6	
6-OHDA	Saline		4
NGF-As	NGF-As	4	
NGF-As+6-OHDA	NGF-As		4

Mice were killed by cervical dislocation and the three tissues under investigation were immediately removed and placed in cold McEwen's solution (NaCl 130.0, KCl 5.6, CaCl₂ 2.1, NaH₂PO₄ 1.2, NaHCO₃ 24.9, glucose 11.1, sucrose 13.1 mM; gassed with 5% carbon dioxide in oxygen; McEwen, 1956).

Isolated atria

Adherent tissue was carefully dissected away from both atria which were then bathed in McEwen's solution (containing 0.5 µg/ml atropine sulphate) at 35.5°C; muscle activity was monitored isometrically through a Statham transducer (model UC3 with microscale accessory) and displayed on a Devices M2 recorder. After a 30 min equilibration period, chronotropic responses were elicited at 10 min inter-

vals by applying 50 shocks (0.2 ms duration, supramaximal strength 40 V) at each rate, via parallel platinum wire electrodes in the following order: 2, 5, 10, 25 or 50 Hz. The responses were measured by counting the individual beats in an 18 s period beginning 6 s after cessation of stimulation and expressing the response as an increase (in beats/min) over pre-stimulation rates. The tissue was then washed by overflow and 15 min later a cumulative concentration-response curve to noradrenaline was determined, each concentration being allowed to remain in contact with the tissue for 60 s before the next increment. The chronotropic response was expressed as the difference (in beats/min) between the resting rate before the curve was started and the maximum rate (the highest value of average rates calculated for consecutive 6 s periods) attained during exposure to each increment. The tissue was then washed periodically by overflow and 30 min later the response to 50 Hz electrical stimulation was redetermined.

Isolated vas deferens

The tissues were mounted (usually in pairs) in a tissue bath in McEwen's solution at 35.5° C between parallel platinum wire electrodes. Contractions (load 200 mg) were recorded through an isotonic transducer (Hannon, Hughes & Letley, 1970) and displayed on a Heathkit chart recorder. Tissues were allowed to equilibrate for 15 min and were then stimulated at 5 min intervals with trains of rectilinear pulses (0.2 ms duration, supramaximal strength 40 V). Two hundred and fifty shocks were applied at various rates in the following order: 50, 50, 50, 2.5, 5, 10, 25, 50 or 100 Hz. The amplification of the recording system was adjusted during the first two periods of stimulation in each experiment. A concentration-response curve to noradrenaline was then determined, each concentration being allowed to remain in contact with the tissue for 30 s; 5 min was allowed to elapse between successive doses which were applied in ascending order of magnitude. The tissue was then washed repeatedly by overflow and the response to 50 Hz stimulation redetermined. All responses are expressed as contractions of the tissue in mm.

Isolated superfused ileum with sympathetic nerves attached

A segment of ileum about 1.5 cm long, located between 5 and 10 cm from the ileo-caecal junction, was suspended in air inside a heated water jacket, the proximal end being attached to a balanced lever system (load 850 mg) and the distal end secured to a glass tissue holder. Segments were chosen such that a mesenteric arterial branch (carrying the periarterial sympathetic nerves) emerged at the proximal end allowing a pull-through bipolar platinum electrode (Burn & Rand, 1960) to be accommodated in the water jacket without interfering with the movements of the tissue. The ileum was continuously superfused with McEwen's solution at 32° C at a rate of 0.5 ml/minute. A second slow stream of solution was allowed to run down the inner surface of the water jacket keeping moist the periarterial nerves. The nerves were stimulated with 800 rectilinear pulses of 0.5 ms duration and supramaximal voltage (15–20 V) at each of a variety of frequencies from 2 to 200 Hz. Frequency-response curves were first determined in each tissue. Subsequently, noradrenaline was applied to the tissue by superfusion of a solution of the drug for 30 s followed by a return to McEwen's solution; changes in temperature or flow rate were thus avoided. A 5 min cycle was used in each experiment. Movements of the ileum were recorded on a Heathkit chart recorder. The upper edge of the peris-

taltic record was used in measuring the responses which were expressed in millimetres. Since the amplification of the recording system remained constant the measurements were comparable throughout the experiments.

Fluorescence histochemistry

Tissue from one mouse of each group was used for histological examination. The left vas deferens, a piece of ileum some 2 or 3 cm from the ileo-caecal junction and cardiac ventricular tissue were prepared for microscopic examination as described by El-Badawi & Schenk (1967) in their 'Method II for noradrenaline'. Briefly, the tissue was frozen rapidly with dry ice and acetone, after which cryostat sections of 10–15 μm thickness were cut at -16°C . Air dried mounted sections were fixed in a formalin/citric acid/Tyrode solution prior to nuclear staining and after further air drying were exposed to formaldehyde vapour at 55°C for 30 minutes. The sections were dehydrated, cleared and mounted in non-drying low fluorescence immersion oil and examined with a Zeiss photomicroscope using ultraviolet illumination. With this technique the clearest fluorescence is observed after about 24 hours. Fading then begins between 7 and 10 days later. The sections were subjectively assessed for fluorescence on the second or third day after preparation.

Drugs

L-Ascorbic acid (BDH), atropine sulphate (BDH), bretylium tosylate (Wellcome), freeze dried nerve-growth-factor antiserum (Horse) (Wellcome: Batch No. BW 85/19/13), guanethidine sulphate (Ciba), 6-hydroxydopamine hydrochloride (Emmanuel), (–)-noradrenaline bitartrate (Koch-Light) and (\pm)-propranolol hydrochloride (ICI). All concentrations are expressed in terms of these salts unless otherwise stated.

Statistical procedures

All results quoted are means \pm standard error of the mean (S.E.M.) and tests for significance were performed with Student's *t* test.

Results

Isolated atria

Spontaneous rates (beats/min) for the four experimental groups were as follows: control, 340 ± 12 ; NGF-As, 319 ± 28 ; 6-OHDA, 312 ± 12 and NGF-As+6-OHDA, 293 ± 51 and none of these resting values was significantly different from the control value ($P > 0.3$). Electrical stimulation at frequencies up to 10 Hz effectively 'drove' the tissues but higher frequencies completely abolished co-ordinated spontaneous activity though this returned immediately the stimulus was stopped. Thereafter, positive chronotropic and inotropic responses developed which both reached a maximum about 18 s after cessation of the stimulus. These responses were blocked by propranolol (0.5 $\mu\text{g/ml}$) and by prolonged treatment with guanethidine (8 $\mu\text{g/ml}$) or bretylium (10 $\mu\text{g/ml}$) and are therefore considered to be a result of activation of adrenergic nerves. Although the heart rate could be measured accurately, the inotropic response was distorted by the poor frequency-response

characteristics of the Devices M2 recorder and therefore only chronotropic responses are reported in this paper.

Control atria showed an increasing chronotropic response as the frequency of stimulation was increased with a maximum at 25 Hz (Fig. 1). A similar effect, though smaller in magnitude, was seen in atria from the NGF-As group. The responses were only significantly different ($P < 0.05$) from those in control tissues at frequencies of 2 and 5 Hz. At higher frequencies there was no significant difference ($P < 0.2 > 0.05$). Atria from the 6-OHDA group showed greatly reduced chronotropic responses which did not increase with increasing frequency of stimulation. The responses were significantly different ($P < 0.02$) from the controls at all frequencies. Combined NGF-As+6-OHDA treatment abolished the chronotropic response to electrical stimulation in nearly all the mice and the mean responses at all frequencies were significantly smaller than those seen in the controls ($P < 0.001$) and in the other two treated groups ($P < 0.05$) (Fig. 1).

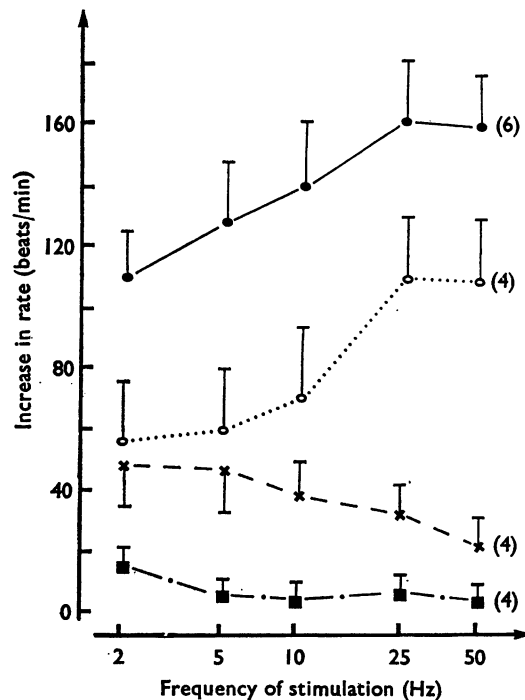


FIG. 1. Mouse atria showing relationship between frequency of stimulation (Hz) and chronotropic response (increase in rate of beat: beats/min; mean \pm S.E.M.) in the following groups, \bullet — \bullet control; \circ — \circ nerve-growth-factor antiserum (NGF-As); \times — \times 6-hydroxydopamine (6-OHDA); \blacksquare — \blacksquare NGF-As+6-OHDA. Figures in parentheses show numbers of tissues contributing to each point. Electrical stimulation at all frequencies consisted of 50 rectilinear pulses each of 0.2 ms duration and supramaximal strength (40 V).

There was a 10–20 fold increase in atrial sensitivity to noradrenaline in the three treated groups compared with the controls, but the maximum possible chronotropic response to noradrenaline was not significantly different ($P > 0.5$) (Fig. 2). In order to provide some measure of the integrity of the sympathetic innervation, the ratio was calculated of the chronotropic response after stimulation at 25 Hz (the optimal frequency) to the maximal chronotropic response to noradrenaline in each tissue.

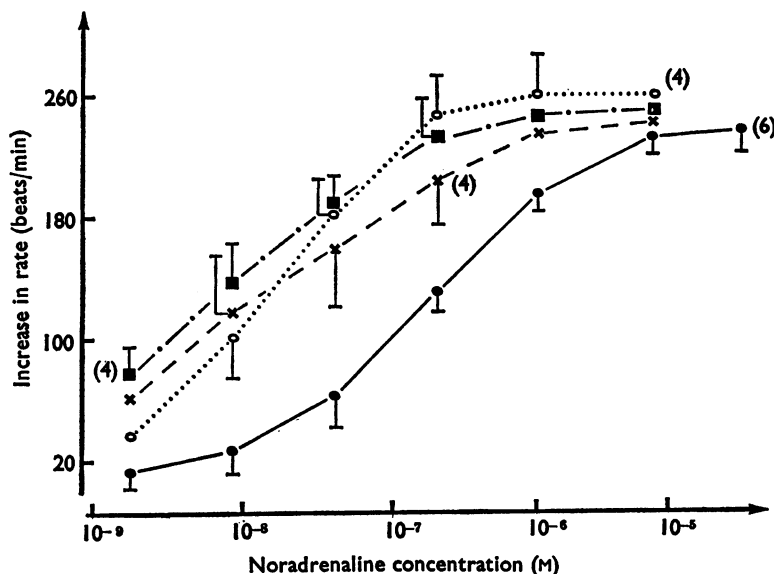


FIG. 2. Mouse atria showing relationship between concentration of noradrenaline (M) and the chronotropic response (increase in rate: beats/min: mean \pm S.E.M.) in the following groups, \bullet — \bullet control; \circ — \circ nerve-growth-factor antiserum (NGF-As); \times — \times 6-hydroxydopamine (6-OHDA); \blacksquare — \blacksquare NGF-As+6-OHDA. S.E.M.s are shown for the control group and for those points which are significantly different ($P < 0.05$) from the controls at the appropriate concentrations. Figures in parentheses show numbers of tissues contributing to each point.

Taking the means of these ratios, in control atria electrical stimulation produced $69 \pm 6\%$ of the maximal chronotropic response to exogenous noradrenaline whilst in the NGF-As group the figure was $45 \pm 8\%$ and was significantly smaller ($P < 0.05$) than the control. 6-OHDA treatment produced a further reduction to $15 \pm 5\%$, a figure significantly less than the two previous groups ($P < 0.001$) and the combined treatment caused still further reduction to $2 \pm 2\%$.

The high concentration of noradrenaline used in the cumulative concentration-response curves sometimes induced irregularities in the spontaneous activity of the atria that persisted for a considerable time; this made redetermination of the effects of 50 Hz stimulation somewhat difficult. However, in those tissues which did not develop irregularities, no marked or consistent differences were noted in the size of the responses to 50 Hz electrical stimulation before and after determination of the concentration-response curve to noradrenaline.

Isolated vas deferens

Transmural stimulation of the vas deferens produced small responses at low frequencies which became larger as the frequency of the applied stimuli was increased (Fig. 3). The frequency-response curves from control and NGF-As groups were not significantly different ($P > 0.05$) at any point but treatment with 6-OHDA, with or without NGF-As, produced a marked reduction in the response to all frequencies ($P < 0.02$) except 2.5 Hz. However, in contrast to the finding in atria, 6-OHDA was equally effective whether given alone or in combination with NGF-As and there was no significant difference in the response from these two groups at any frequency ($P > 0.2$) (Fig. 3).

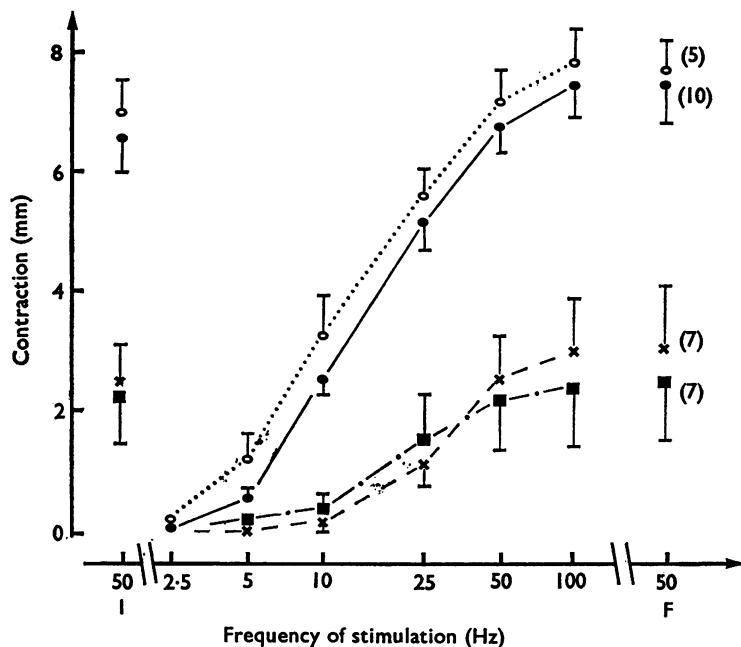


FIG. 3. Mouse vas deferens showing relationship between frequency of stimulation (Hz) and contraction of the tissue (mm; mean \pm S.E.M.) in the following groups, \bullet — \bullet control; \circ — \circ nerve-growth-factor antiserum (NGF-As); \times — \times 6-hydroxydopamine (6-OHDA); \blacksquare — \blacksquare NGF-As+6-OHDA. The responses to an initial (I) and final (F) period of 50 Hz stimulation are also shown. Figures in parentheses show numbers of tissues contributing to each point. Electrical stimulation at all frequencies consisted of 250 rectilinear pulses each of 0.2 ms duration and supramaximal strength (40 V). (The 6-OHDA and NGF-As+6-OHDA groups did not respond to 2.5 Hz and these points have been omitted for simplicity, as have the S.E.M.'s for the other two groups at this frequency which fell within the area of the points.)

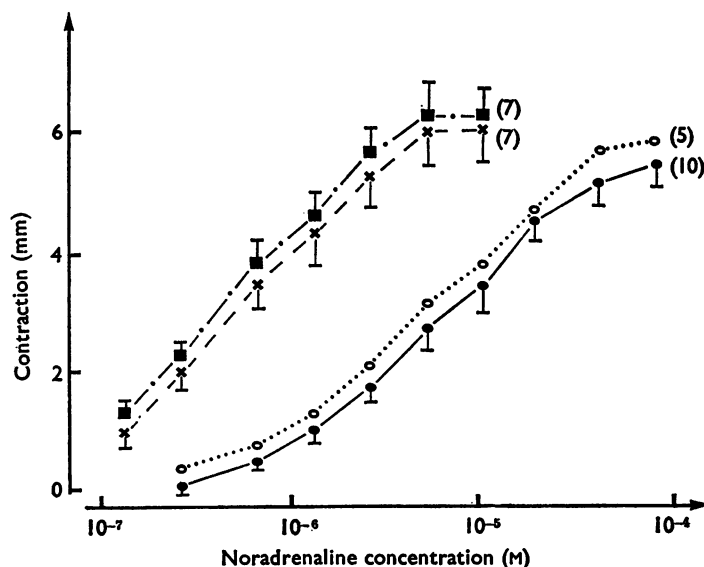


FIG. 4. Mouse vas deferens showing relationship between concentration of noradrenaline (M) and contraction of the tissue (mm; mean \pm S.E.M.) in the following groups, \bullet — \bullet control; \circ — \circ nerve-growth-factor antiserum (NGF-As); \times — \times 6-hydroxydopamine (6-OHDA); \blacksquare — \blacksquare NGF-As+6-OHDA. S.E.M.s are shown for the control group and for those points which are significantly different ($P < 0.05$) from the controls at the appropriate concentrations. Figures in parentheses show numbers of tissues contributing to each point.

The noradrenaline concentration-response curves showed that the sensitivity of the vas deferens was increased some 15- to 20-fold in 6-OHDA and NGF-As+6-OHDA groups but that NGF-As alone failed to produce any significant alteration in noradrenaline sensitivity when compared with controls (Fig. 4). The maximum responses to noradrenaline which could be elicited from each of the four groups of tissues were not significantly different from each other ($P>0.3$). A similar expression of nerve fibre integrity has been used for these tissues as was described for the atria: thus, electrical stimulation at 100 Hz produced $147 \pm 11\%$ of the maximal response to noradrenaline in the control tissues and after NGF-As treatment ($139 \pm 9\%$) there was no significant change in this figure ($P>0.5$). Compared with controls, significantly smaller percentages ($P<0.001$) were seen in 6-OHDA and NGF-As+6-OHDA groups ($40 \pm 11\%$ and $39 \pm 10\%$ respectively) but there was no significant difference between these two figures ($P>0.8$).

In all four groups, the response to 50 Hz stimulation showed some tendency to increase as the experiment progressed but in no case was this increase marked or significant ($P>0.3$) (Fig. 3).

Isolated ileum with sympathetic nerves attached

Pendular movements occurred in all intestinal preparations. In control tissues, sympathetic nerve stimulation caused a reduction in both the size of these movements and in the tone of the preparation and stimulation with 800 shocks was sufficient to produce a plateau response at all frequencies. Generally, stimulation at 2, 5 or 10 Hz caused a reduction in pendular movements with little effect on tone, but at 20 Hz and higher, abolition of the spontaneous movements occurred and was accompanied by a considerable relaxation. Reproducible responses could be elicited for several hours and in no case was there any deterioration during the course of the experiment (2 to 2.5 hours).

Compared with controls, preparations from the NGF-As group responded less well to nerve stimulation. The most marked differences were at frequencies of 20 Hz and lower ($P<0.05$) and in two experiments a motor response was seen on stimulation at 5 Hz (Fig. 5). Tissues from the 6-OHDA group usually contracted when stimulated at 2 to 20 Hz and sometimes the pendular movements increased in size. The response was not always maintained however, and was, in some preparations, followed by a small relaxation when electrical stimulation ceased. Essentially similar effects were seen in the NGF-As+6-OHDA group (Fig. 5). In all cases the motor responses were abolished when the tissue was superfused with atropine sulphate ($0.1 \mu\text{g/ml}$) at the end of the experiment.

Superfusion of the ileum from any group with noradrenaline produced effects similar to nerve stimulation in control tissues. Concentration-response curves are shown in Fig. 6 from which it may be seen that in the three treated groups the response to the highest concentration was smaller than that in the control group.

Fluorescence-histochemistry

In control vas deferens a bright green fluorescent network with an obvious beaded appearance typical of varicosities in noradrenergic nerves was seen to pervade the smooth muscle layers; in some areas the fluorescence was very intense. NGF-As treatment did not appear to alter the intensity of the fluorescence but did reduce

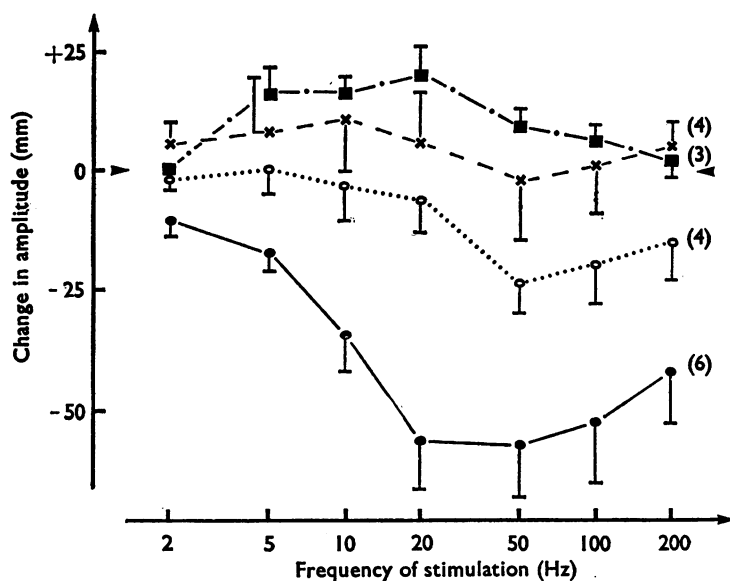


FIG. 5. Superfused mouse ileum with sympathetic nerves attached showing relationship between frequency of stimulation (Hz) and response (mm of relaxation or contraction: mean \pm S.E.M.) in the following groups, ●—● control; ○---○ nerve-growth-factor antiserum (NGF-As); ×---× 6-hydroxydopamine (6-OHDA); ■---■ NGF-As+6-OHDA. Figures in parentheses show numbers of tissues contributing to each point. Electrical stimulation at all frequencies consisted of 800 rectilinear pulses each of 0.5 ms duration and supramaximal voltage (15–20 V).

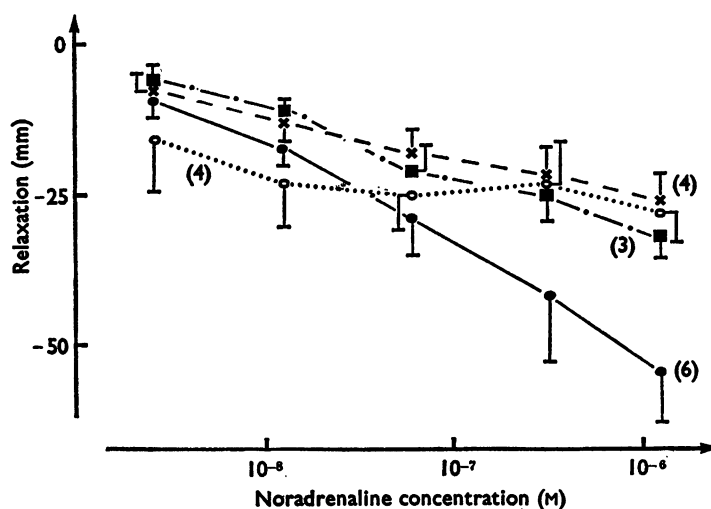


FIG. 6. Superfused mouse ileum with sympathetic nerves attached showing relationship between concentration of noradrenaline (M) superfusing the tissue for 30 s and the response (relaxation in mm; mean \pm S.E.M.) in the following groups, ●—● control; ○---○ nerve-growth-factor antiserum (NGF-As); ×---× 6-hydroxydopamine (6-OHDA); ■---■ NGF-As+6-OHDA. Figures in parentheses indicate numbers of tissues contributing to each point.

the area in which the green beaded network was seen. In both 6-OHDA and NGF-As + 6-OHDA groups there was a further reduction of areas where fluorescence could be detected and an apparent diminution in its intensity. The beaded appearance was lost and occasional spots of yellowish fluorescence were observed apparently deposited in the muscle layers and dissociated from any fibrous network.

No such gradation of effect was obvious in cardiac or intestinal preparations. Although a few fine green fluorescent fibres were seen in the control myocardium no specific fluorescence was seen in the ventricles of mice taken from the other three groups. The intestinal sections invariably contained large areas of bright yellow fluorescence (probably associated with biogenic amines other than noradrenaline) effectively masking any green fluorescent network which might have been present.

Discussion

In mice treated with nerve-growth-factor antiserum (NGF-As), 6-hydroxydopamine (6-OHDA) or a combination of these agents (NGF-As + 6-OHDA) adrenergic nerve function was clearly reduced in all three tissues investigated with the exception of vas deferens where NGF-As given alone caused no change. The relative effectiveness of the three treatments in atria and intestine was in the order NGF-As + 6-OHDA > 6-OHDA > NGF-As. The absolute effectiveness is more difficult to assess since the concomitant increase in the sensitivity of the tissue to exogenous (and presumably endogenous) noradrenaline means that activation of a small number of functional nerve fibres could elicit a large response from the tissue. For example, the responses of vasa deferentia from the 6-OHDA group to varying frequencies of stimulation were about one-fifth as large as the control counterparts. Had no change been seen in the sensitivity of the tissue to noradrenaline, this might have been taken to indicate an 80% reduction in adrenergic nerve function. However, there was a concomitant increase in sensitivity to exogenous noradrenaline of about 20 fold and if a similar increase in sensitivity to endogenous noradrenaline is assumed, then an estimate of 99% inhibition of normal nerve function may be nearer the truth. Fluorescence-histochemistry gives little help in deciding which of these figures is more nearly correct since this technique, whilst giving an indication of the noradrenaline content of the tissue, cannot show the functional availability of the transmitter and it is well known that tissue noradrenaline content is not directly related to nerve function (Swedin, 1971; Wakade & Krusz, 1972). However, it is evident that although a high degree of sympathectomy may have been achieved it was by no means complete unless some non-adrenergic neurones accounted for the residual responses.

In both vas deferens and atria the responses to nerve stimulation differed in magnitude according to the group to which the mice belonged. Although the forms of the frequency-response curves were unchanged in vas deferens after the different treatments, the atria from both groups which had received 6-OHDA produced frequency-response curves quite different in shape from those of the other two groups with the maximum effects being shown at 2 Hz stimulation instead of 25 Hz. It is possible that these differences are artefactual since the periods of electrical stimulation from which the frequency-response curves were drawn were applied in a fixed order working from low frequencies up to high frequencies. Any residual transmitter could therefore be largely utilized during the low frequency stimulation and responses at high frequencies would be further depressed.

There were both quantitative and qualitative differences between intestinal preparations from different groups in response to sympathetic nerve stimulation. After NGF-As alone, a diminished relaxation was seen, whilst after 6-OHDA, alone or in combination with NGF-As, the lower frequencies of stimulation usually produced a motor response or, occasionally, a very small reduction in tone. Since the motor response was inhibited by atropine it was probably mediated through the release of acetylcholine and was possibly masked in normal tissues by the relaxation caused by a greater release of endogenous noradrenaline. This revealed response could be due to the existence of distinct cholinergic nerves (morphologically either sympathetic or parasympathetic) running periarterially or alternatively to overflow of acetylcholine from a cholinergic link in adrenergic nerves (Burn & Rand, 1965; Ferry, 1966; Burn, 1971). The latter explanation seems unlikely since it is probable that the inhibition of sympathetic function after 6-OHDA-treatment is due to destruction of adrenergic nerves which would then be expected to lose the acetylcholine stores proposed in the cholinergic link hypothesis. Although we have no direct electron-microscopical evidence that destruction of adrenergic nerves has taken place in the intestine in these experiments, this action of 6-OHDA has been demonstrated in a variety of tissues and species at comparable dose levels (Furness *et al.*, 1970; Malmfors & Thoenen, 1971). Compatible with the destruction of adrenergic nerves having taken place is the observation that in all three tissues the response to electrical stimulation was not markedly potentiated after concentration-response curves to noradrenaline had been determined (i.e. after effective incubation of the tissues with noradrenaline), though potentiation might be expected if 6-OHDA had merely depleted nerves of noradrenaline.

Levi-Montalcini & Booker (1960) in their early work in mice showed that NGF-As was able to produce a permanent, uniform and virtually complete sympathetomy judged by the destruction of cell bodies of post-ganglionic neurones in several different sympathetic ganglia. The loss of sympathetic nerve function seen in our NGF-As-treated mice was by no means complete; indeed, 6-OHDA produced a more marked impairment. No examination of ganglia was made in the present experiments and so two alternatives can be proposed to account for this discrepancy: either there is no direct association between loss of ganglion cells and functional changes in the innervated organ or a smaller proportion of cells was damaged with the present schedule of treatment than has been observed previously with different batches of NGF-As. The pattern of impairment of sympathetic function in these mice is similar to that seen with NGF-As in the rat where, for instance, Zaimis, Berk & Callingham (1965) showed that the endogenous catecholamine content of the vas deferens and its ability to accumulate labelled noradrenaline were scarcely changed at a time when considerable reductions occurred in other organs. To our knowledge no combined study has been made of ganglion histology, tissue catecholamine content, uptake of labelled noradrenaline and nerve function in the mouse after NGF-As but, until such diverse measurements can be correlated, it remains debatable whether there are real species differences or whether all the differences simply depend on the effective dose of NGF-As.

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