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THE EFFECT OF PITHING AND OF NERVE STIMULATION ON THE DEPLETION OF NORADRENALINE BY RESERPINE IN THE RAT ANOCOCCYGEUS MUSCLE AND VAS DEFERENS

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1 The depletion of noradrenaline (NA) in the rat anococcygeus muscle and vas deferens by reserpine and the effect on this of the abolition of nerve activity by pithing and reinforcement of nerve activity by stimulation of the spinal cord outflows has been studied.

2 NA depletion of the anococcygeus and vas deferens measured 24 h after reserpine was similar and was related to dose. The heart was depleted faster than the two smooth muscle tissues.

3 In the absence of reserpine neither abolition of nerve activity by pithing nor its reinforcement by nerve stimulation had a detectable influence on NA content of the anococcygeus or vas deferens.

4 In rats given reserpine (200 μg/kg), increasing nerve activity by spinal stimulation significantly increased NA depletion in both the anococcygeus and the vas deferens when compared with animals pithed but not stimulated. These results confirm that nerve impulse traffic can be an important factor in determining the rate of depletion of NA by reserpine.

5 The mechanical response to nerve stimulation in both the vas deferens and anococcygeus was resistant to quite severe depletion of their NA content, with the exception of the initial fast component of the response in the vas. The implications of these results for motor transmission in the vas deferens are discussed.

Introduction

Most adrenergically innervated peripheral organs, including the heart, are depleted of their noradrenaline (NA) by reserpine to a similar degree (Bertler, 1961). Exceptions to this are the seminal vesicles and vas deferens which are relatively resistant to depletion compared with other tissues (Sjöstrand & Swedin, 1968).

Two possible explanations of this resistance are that the rate of depletion is dependent on the firing frequency in the nerves and, therefore, reduced in the intermittently active nerves of the vas deferens and seminal vesicles or that the 'short' adrenergic neurones which are found in the seminal vesicles and vas deferens and have their cell bodies located close to the target organ (Owman & Sjöstrand, 1965; Sjöstrand, 1965) are less easily depleted than the 'long' adrenergic neurones found elsewhere (Sjöstrand & Swedin, 1968).

The first hypothesis is supported by the finding that the rate of depletion by reserpine in various organs is reduced if the impulse flow is reduced by decentralization of the adrenergic nerves before reserpine (Holzbauer & Vogt, 1956; Hertting, Potter & Axelrod, 1962; Benmiloud & Euler, 1963; Sedvall, 1964) or administration of ganglion blocking drugs (Kärki, Paasonen & Vanhakartano, 1959; Mirkin, 1961; Hertting *et al.*, 1962).

The second explanation is suggested by the evidence that 'short' adrenergic neurones have a lower sensitivity to 6-hydroxydopamine (Malmfors & Sachs, 1968) as well as to reserpine, react differently to immunosympathectomy (Hamberger, Levi-Montalcini, Norberg & Sjöquist, 1965; Zaimis, Berk & Callingham, 1965; Iversen, Glowinski & Axelrod, 1966) and their transmitter granules when isolated have different properties compared to those from splenic nerve (Euler & Lishajko, 1966; Stjarne & Lishajko, 1966).

We have investigated these hypotheses in the rat vas deferens and anococcygeus muscles. The anococcygeus receives a dense adrenergic innervation uniformly distributed throughout the muscle as in the vas deferens (Gillespie, 1972) but unlike the vas deferens the neurones are conventionally 'long' (Gillespie & McGrath, 1973). The effect of

nerve impulse traffic was investigated by comparison of the rate of NA depletion by reserpine in animals in which all efferent nerve impulses were abolished by pithing with the rate of depletion when this impulse traffic was raised to its maximum by artificial nerve stimulation. For this purpose the pithed rat preparation in which a shielded pithing wire is used to stimulate selected levels of the spinal outflow was used (Gillespie, MacLaren & Pollock, 1970). Reserpine was given 3 h before pithing and tissues taken for assay 3 h after pithing, i.e. after 6 h of reserpine action. Artif. al stimulation of the spinal outflow was for the last 2 h of this 6 h period. The effect of pithing alone and pithing plus stimulation in the absence of reserpine was also examined.

A preliminary account of these results has been published (Gillespie & McGrath, 1972).

Methods

Male Wistar rats (250-300 g) were used. Reserpine was given intraperitoneally in an aqueous solution containing 0.4% ascorbic acid.

Relation of noradrenaline depletion to dose of reserpine

In these experiments the effects of different doses of reserpine were examined 24 h after administration of the drug. Groups of six animals were given reserpine in doses of 50, 100, 200 or 1000 μg/kg. After 24 h the rats were killed by a blow on the head, bled and the vas deferens and anococcygeus muscles removed for assay of their NA contents.

Time course of noradrenaline depletion by reserpine

The vas deferens and anococcygeus muscles were removed from groups of six rats and NA assayed at 3, 6, 12 and 24 h after intraperitoneal administration of reserpine, 200 μg/kg. This dose had been found to produce a marked but submaximal depletion of NA in both tissues at 24 h, a degree of depletion which would allow detection of an alteration following interruption or stimulation of nerve impulse traffic to the tissues. At 3 and 6 h after reserpine the heart, as well as the anococcygeus and vas deferens, was taken for NA assay.

The effect of pithing and of pithing plus stimulation on the noradrenaline depletion induced by reserpine

In these experiments the autonomic nerves were stimulated in the spinal canal by a shielded, moveable stainless steel electrode which was also used to pith the animal. Before pithing, the rats were anaesthetized with a mixture of nitrous oxide and halothane. A protruding length of 10 mm of unshielded electrode was used to stimulate the autonomic preganglionic fibres between L1 and L3, a region which includes the fibres to both the anococcygeus and the vas deferens. Ten second trains of pulses at a frequency of 30 Hz, 1 ms duration and supramaximal voltage were given every 1.5 min for 2 hours. Intravenous pancuronium bromide (1 mg/kg i.v.) was given to prevent skeletal muscle twitching. The mechanical responses of the two anococcygeus muscles jointly and of one vas deferens were each recorded isometrically with Grass FTO3 transducers and displayed on a Grass polygraph. Tension in the vas was recorded from the freed epididymal end with the prostatic end as the fixed point. The technique of recording the response of the anococcygeus has already been described (Gillespie & McGrath, 1973). The systemic blood pressure and heart rate were also recorded.

Experimental design to examine the effects of pithing and of pithing plus nerve stimulation on the rate of reserpine-induced depletion of noradrenaline

The protocol for these experiments is shown in Table 1. Four groups of rats were used. The first group was pithed only (group 1), a second group pithed and the autonomic outflow stimulated (group 2), a third group was given reserpine and then pithed (group 3) and a fourth group given reserpine, pithed and the autonomic outflow stimulated (group 4). The preparation of the muscles for recording occupied the first hour after pithing so that nerve stimulation was applied for the 2 h period beginning 1 h after pithing. Reserpine was given 3 h before pithing the animals. All tissues were removed for assay 3 h

Table 1 Experimental design to examine the effects of pithing or pithing plus nerve stimulation on the rate of noradrenaline (NA) depletion by reserpine

Group No.	*Reserpine (200 µg/kg i.p. at 0 h)*	*Pithed (at 3 h)*	*Stimulated (4 h – 6 h)*
1	–	+	–
2	–	+	+
3	+	+	–
4	+	+	+

Tissues were removed for NA assay at 6 hours.

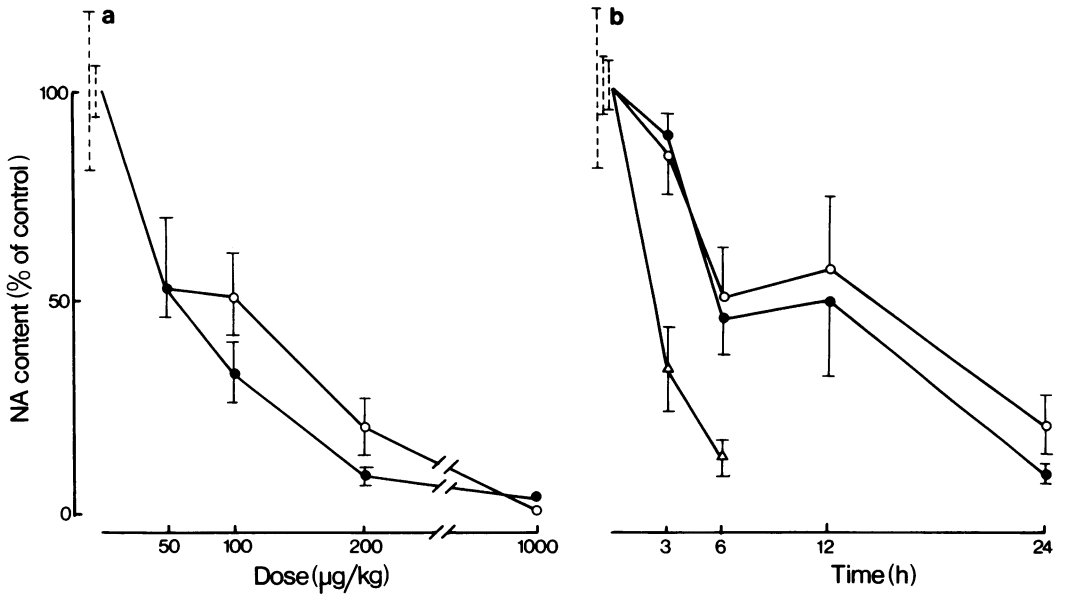

Fig. 1 (a) Effect of varying the dose of reserpine on the noradrenaline (NA) content of rat anococcygeus and vas deferens after 24 hours. (b) Time course of NA depletion of the rat anococcygeus, vas deferens and heart following a single dose of reserpine (200 μg/kg i.p.). NA content is expressed as a percentage of the mean value from untreated controls for each tissue. (•) anococcygeus; (○) vas deferens; (△) heart. Solid vertical lines represent s.e. mean; dashed vertical lines represent s.e. mean for the untreated controls; from left to right, anococcygeus, vas deferens, heart.

after pithing, i.e. 6 h after giving reserpine in groups 3 and 4.

Assay of tissue noradrenaline

Tissues from pairs of similarly treated animals were pooled for assay. The tissues were weighed, frozen in liquid nitrogen and then pulverized in a precooled stainless steel mortar and pestle. The powder was extracted with 0.4 M perchloric acid and the noradrenaline in the extract estimated fluorometrically by the trihydroxyindole method of Euler & Lishajko (1961), after adsorption on to alumina and elution by 0.25 M acetic acid. Fluorescence intensity was measured with an Aminco-Bowman spectrofluorimeter. Duplicate recoveries of 1 μg pure NA applied to alumina columns were carried through in each experiment. Recoveries ranged from 70-85% over all experiments but never differed by more than 2% in a single experiment. The NA content was corrected on the basis of the mean recovery in each experiment and expressed as μg NA/g tissue. The mean NA content in each of the four groups of experiments was then expressed as a percentage of the normal NA content for that tissue based on assays of tissue from ten normal rats (5 assays). Statistical comparison between groups was made with Student's *t* test.

Drugs

Drugs used were pancuronium bromide (Organon) and reserpine (crystalline, Koch-Light).

Results

Effect of reserpine dose on the tissue noradrenaline

In untreated rats the mean concentration of NA in the anococcygeus was 2.56 ± 0.48 μg/g (n = 5), in the vas deferens 15.04 ± 0.86 μg/g (n = 5) and in the heart 1.13 ± 0.05 μg/g (n = 3).

Figure 1a shows the changes in the NA concentration in the anococcygeus muscle and the vas deferens 24 h after the injection of various doses of reserpine. The highest dose (1000 μg/kg) caused, in both tissues, almost complete depletion to 3% and 1% of the normal values. After 200 μg/kg, 9 ± 2% was left in the anococcygeus

and 20 ± 7% in the vas. There was no statistically significant difference in the NA depletion at any dose level between the two tissues ($P > 0.05$).

Time course of the noradrenaline depletion by reserpine (200 μg/kg)

Figure 1b shows the time courses of the percentage depletion of NA in the heart, anococcygeus and vas deferens after reserpine (200 μg/kg i.p.). The rates of depletion of the anococcygeus and vas deferens were similar and slower than the rate of depletion in the heart. After 6 h the NA content of the heart was 12 ± 4% ($n = 3$), whereas that of the anococcygeus was still 45 ± 8% ($n = 7$) and that of the vas deferens 50 ± 8% ($n = 7$) of their respective controls. This dose of 200 μg/kg was chosen as suitable for the investigation of nerve impulse traffic on the rate of NA depletion since it produced within 6 h a level of depletion of NA appropriate to displaying either a reduction or increase in depletion rate by procedures interfering with or reinforcing nerve activity.

Effect of pithing and of pithing plus nerve stimulation in animals not treated with reserpine

Interruption of spontaneous neuronal activity by pithing alone (group 1) or increase in neuronal activity by a subsequent 2 h period of intermittent nerve stimulation (group 2) produced no significant changes in the NA content of the anococcygeus or vas deferens compared either with control rats or comparing the two groups with one another (Table 2).

Effect of pithing and nerve stimulation in reserpine-treated animals

In rats pretreated 3 h earlier with reserpine, 200 μg/kg (group 3), pithing resulted in a non-significant reduction in the depletion of NA in both the anococcygeus and vas deferens compared with unpithed controls. In group 4 where in addition the autonomic outflows were stimulated for 2 h the depletion of NA was greater than in controls though again this difference was not statistically significant. When groups 3 and 4 were compared, however, there was a significant difference in NA depletion in both the anococcygeus muscle and the vas deferens.

Effect of reserpine on the mechanical response to nerve stimulation of the vas deferens and anococcygeus

Since the tension responses in both anococcygeus and vas deferens were recorded it was possible to correlate the changes in NA content with changes in the mechanical response. In control animals the 10 s periods of stimulation at 30 Hz produced maximal contractions which in the anococcygeus consisted of a single smooth contraction and in the vas deferens of the typical two phase contraction, an initial quick twitch reaching its maximum in 1.5 s then declining to be followed by a slower secondary contraction reaching a plateau after 8-10 seconds. On average the initial twitch exceeded the secondary response by 20 ± 2% ($n = 15$). Repeated stimulation at 1.5 min intervals over a 2 h period resulted in a slight decline in both components of the response in the vas deferens but particularly affecting the initial twitch. In the anococcygeus the response increased slightly over the first 15 min and then remained constant for the rest of the 2 h period. Reserpine (200 μg/kg) had no effect on the response of the anococcygeus or on the secondary response in the vas deferens. The initial twitch component of the response in the vas deferens, though normal in amplitude when first elicited, rapidly diminished with repeated stimulation and after 15-20 min had quite disappeared.

Table 2 Effects of pithing or pithing plus nerve stimulation on the rate of noradrenaline (NA) depletion by reserpine on the anococcygeus muscle and vas deferens of the rat

Group	No. of rats	Mean NA content (± s.e. mean) as % of control: Anococcygeus		Vas deferens	
1. Pithed	6	128 ± 8	$P > 0.05$	95 ± 4	$P > 0.05$
2. Pithed + stim	6	101 ± 13		82 ± 6	
3. Reserpine + pithed	8	58 ± 14	$0.025 > P > 0.0125$	64 ± 7	$0.0125 > P > 0.01$
4. Reserpine + pithed + stim	8	18 ± 6		32 ± 5	

The NA content is expressed as a percentage of the mean values from untreated control rats for each tissue. Reserpine (200 μg/kg i.p.) was given 3 h before pithing, and tissues removed for NA assay 3 h after pithing. In groups 2 and 4 the spinal outflows were stimulated intermittently between the 4th and 6th hour after giving reserpine

Discussion

The similarity in the extent and rate of depletion of NA by reserpine in the rat anococcygeus, which has conventionally long adrenergic neurones (Gillespie & McGrath, 1973), and the rat vas, which has short adrenergic neurones, would appear to eliminate the length of neurone as a significant factor in determining resistance to the depleting action of reserpine. However, the results are consistent with the extent of nervous activity having an important influence on depletion. For example, the main difference between the heart and the other two tissues is in the rate of depletion rather than the extent of depletion eventually achieved. It is known that there is a continuous discharge in the sympathetic nerves to the heart (Bronk, Ferguson, Margaria & Solandt, 1936) and that this is reflexly increased as reserpine lowers systemic blood pressure (Iggo & Vogt, 1960). We have no information on the presence of spontaneous activity in the nerves to the vas or anococcygeus nor are we aware of such information in the literature but in anaesthetized rats neither tissue shows spontaneous activity *in situ* suggesting that neuronal activity, if present, must be slight.

These differences in spontaneous neuronal activity are at least consistent with the concept that the rate of depletion varies with nerve impulse traffic. Direct support for this is found in a comparison between the extent of NA depletion 6 h after giving reserpine in animals in which impulse traffic is arrested and those in which it was enhanced by intermittent artificial stimulation. Reserpine in the absence of impulses produced a depletion to 58% of normal in the anococcygeus and 64% in the vas and with artificial stimulation a depletion to 18% of normal in the anococcygeus and 32% in the vas; the difference between these values for each tissue was statistically significant. These results are in agreement with previous work on the effect of eliminating nervous activity either by decentralization or by ganglion blocking agents on the depletion of NA by reserpine (Holzbauer & Vogt, 1956; Kärki *et al.*, 1959; Hertting *et al.*, 1959; Mirkin, 1961; Benmiloud & Euler, 1963). One conflicting report is that of Sjöstrand & Swedin (1968) who found that pre-ganglionic hypogastric section had no effect on the NA depletion of the vas deferens by reserpine. This they interpreted as meaning that nerve impulse traffic was not an important factor. However, an alternative explanation could be that in tissues where impulse traffic is low it is unlikely that much effect will be seen by its abolition. This problem is overcome in the present experiments by contrasting the depletion where nerve activity has been stopped with that where nerve activity has been artificially increased.

The motor innervation of the anococcygeus is undoubtedly adrenergic but doubt has been raised as to the mediator of contraction in the vas deferens (Ambache & Zar, 1971). Part of the difficulty may be in the failure to distinguish between the two components of the vas deferens response in describing results. The present experiments show that the initial twitch is sensitive to depression by even small doses of reserpine and rapidly disappears with repeated stimulation. Von Euler (1970) using short trains of pulses which elicit mainly the twitch response has reported a similar ability of reserpine to abolish the motor response in the guinea-pig vas deferens *in vitro*. These results suggest that this twitch component at least is adrenergic and that the store of NA on which it is based may be small. In contrast the entire response of the anococcygeus muscle and the secondary component of the response in the vas are resistant in the short term (6 h) to even large doses of reserpine even though the NA stores are substantially reduced. When the stores are almost completely exhausted at 24 h there is a reduction in both the anococcygeus response and the secondary component of the response in the vas. These results suggest that both components of the motor response in the vas deferens may have an adrenergic basis but the safety margin for effective neuroeffector transmission for the twitch is lower and more readily encroached on by reserpine.

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