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In vitro denervation of the rat vas deferens through hypothermic storage

N.H. Jurkiewicz, *A.G. Garcia & [1]A. Jurkiewicz

Department of Pharmacology, Escola Paulista de Medicina, Caixa Postal 20372, São Paulo 04034, Brazil and *Department of Pharmacology, Facultad de Medicina, Universidad Autónoma de Madrid, Arzobispo Morcillo s/n, Madrid, Spain

1 The rat vas deferens was excised, stored at 4–6°C and tested after 24, 48, 72 or 96 h for its contractile activity and for the presence of innervation.

2 The maximal contractile capacity of the vas, tested through cumulative concentrations of barium chloride (3×10^{-2} M) was progressively reduced from about 110 mm to about 63 mm after 72 h, without further decay after 96 h. Spontaneous rhythmic contractions were practically absent.

3 A loss of endogenous pools of catecholamines was indicated by four parameters: (a) a decline of about 80% after 24 h and of more than 95% after 48 h of the contractile effect of the indirect sympathomimetic agonist tyramine; (b) a fall of about 20%, 50% and 85% on the concentration of noradrenaline, respectively after 24, 48 and 72 h; (c) a fall of about 25% and 90% after respectively 24 and 48 h, of the activity of dopamine-β-hydroxylase (DBH); (d) a decline of noradrenaline-induced histofluorescence on cross sections of the vas.

4 A loss of neuronal uptake capacity was indicated by: (a) a progressive variation of the apparent affinity for adrenaline, expressed as pD_2 values, that increased by about 1.5 log units (corresponding to a 30 fold potentiation) after 72 h, and (b) a reduction of the ability of cocaine to potentiate the contractile effects of adrenaline.

5 The pD_2 values for barium chloride, 5-hydroxytryptamine (5-HT) and histamine were not significantly changed, while the corresponding value for acetylcholine was slightly but significantly reduced by about 0.8 log units.

6 The maximal heights of concentration-response curves for noradrenaline, acetylcholine, histamine and 5-HT were reduced by 42–66% in relation to controls. However, when this reduction was measured in relation to the corresponding barium effect, by means of the relative responsiveness ratio (ρ), a small though significant increase was observed for noradrenaline, and a fall for the other drugs.

7 It is concluded that: (1) the values for the various biochemical and pharmacological parameters decline at different rates, though revealing altogether that denervation is completed by at least 85% after 72 h of hypothermic storage; (2) two of the results, i.e., the lack of spontaneous rhythmic contractions and the lack of increased contractile effects for acetylcholine, 5-HT and histamine, indicate that in these conditions the vas is devoid of the so-called nonspecific signs of denervation.

Keywords: Denervation; vas deferens; dopamine-β-hydroxylase; noradrenaline; acetylcholine; barium; 5-hydroxytryptamine; hypothermic storage

Introduction

It is known that adrenergic innervation can influence contractile effects induced by drug-receptor interactions in rat vas deferens (Jurkiewicz *et al.*, 1977; 1991). Thanks mainly to surgical denervation (Kasuya *et al.*, 1969; Birmingham, 1970), a number of biochemical, pharmacological and histochemical alterations could be ascribed to the degeneration of nerve terminals in this organ: a decrease of the catecholamine-storing vesicles can be detected by a decrease of the activity of dopamine-β-hydroxylase (DBH), an enzyme that is known to be present only in these vesicles (Klein, 1982); a decrease in the pools of neurotransmitter, by a fall of noradrenaline (NA) concentration, and by a decrease in the amplitude of contractions induced by the indirect agonist tyramine; a decrease of neuronal uptake, by an increase of the apparent affinities for noradrenaline and adrenaline, and by a decrease of the ability of cocaine to potentiate the contractile effects of these agonists; in addition, two other effects, the causes of which are not totally known, have been observed in denervated preparations: a non-specific increase of the maximal effect of some agonists, that can be accompanied by a leftward shift of concentration-response curves (Kasuya *et al.*, 1969; Westfall *et al.*, 1975; Kasuya & Suzuki, 1978), and the presence of spontaneous rhythmic contractions (Lee *et al.*, 1975; Goto *et al.*, 1976). Several of these changes have also been observed after other denervation procedures in vas, as for instance after treatment with 6-hydroxydopamine (Westfall & Fedan, 1975), bretylium (Murdock *et al.*, 1977), colchicine (Wakade, 1978; Goto *et al.*, 1979) and vinca alkaloids (Wakade, 1979).

In a pioneering publication, Martins & Valle (1939) reported that the vas deferens maintains its responsiveness to drugs for as much as 13 days, if stored in a regular refrigerator up to the moment of the experiment. In addition, they reported that responses to adrenaline were almost always the last to disappear, in relation to other agonists. Since this could indicate a potentiation of adrenaline effects due to nerve degeneration, experiments were carried out on the rat vas deferens submitted to hypothermic storage, to examine its innervation characteristics. This examination was performed by checking the presence, or not, of the forementioned changes described for the denervated vas.

[1] Author for correspondence.

Methods

Vas deferens excision and hypothermic storage

Wistar male rats of our own colony, BAW2 (Festing, 1980) were used. After laparotomy under ether anaesthesia, one vas deferens was cleared of surrounding tissues, and removed as previously described (Jurkiewicz *et al.*, 1977). The organ was placed in a Petri dish between two cotton layers embedded in nutrient solution of the following composition (mM): NaCl 138, KCl 5.7, $CaCl_2$ 1.8, NaH_2PO_4 0.36, $NaHCO_3$ 15.0 and glucose 5.5, prepared in distilled water. The covered Petri dish was placed in a regular refrigerator for hypothermic storage (about 4–6°C). After 24, 48, 72 or 96 h, vasa deferentia were placed in gassed nutrient solution to be processed for biochemical, pharmacological and histological analysis. Usually the contralateral vas deferens was excised under ether anaesthesia shortly before the experiment, and used as a control. In some experiments normal vasa were also used as controls.

Tissue content of noradrenaline

NA content was estimated following the fluorometric method of Anton & Sayre (1962).

Assay of tissue dopamine-β-hydroxylase activity

Tissue DBH activity was assayed according to the procedure described by Molinoff *et al.* (1971). Treated or control vasa deferentia were individually homogenized in 25 volumes (w/v) 0.005 M cold Tris-HCl buffer, pH 7.4, containing 0.2% Triton-X-100. The homogenates were centrifuged at 27,000 *g* at 2°C and the enzyme activity assayed in 200 µl aliquots of the supernatants. Several concentrations of $CuSO_4$ were tested to obtain adequate inactivation of the endogenous inhibitors of the enzyme. Maximal DBH activities were repeatedly found at 1.6×10^{-4} M (final concentration of $CuSO_4$ in the reaction mixture). The optimal pH for maximal DBH activity was 5.0. Results are expressed as nmol of octopamine formed from tyramine per hour, per g of wet tissue weight (nmol h^{-1} g^{-1}).

Localization of noradrenaline by histofluorescence

Cooled and control vasa deferentia were frozen, freeze-dried, treated with paraformaldehyde, infiltrated *in vacuo* with paraffin, and cut for examination on a fluorescence microscope, according to the technique of Falck (1962). The distribution and intensity of fluorescence were measured by eye, through an arbitrary score, by comparison with the green fluorescence of normal preparations. The background fluorescence had a homogeneous distribution, pallid yellow colour, low intensity, and could be easily differentiated from that due to NA.

Concentration-response curve studies

Cooled and control organs were washed with nutrient solution, weighed and mounted in 10 ml organ chambers containing continuously aerated nutrient solution at 30°C. Isotonic contractions were recorded on smoked drums with frontal levers, with 6 fold amplification and a load of 0.5 to 1 g.

After an initial equilibration period of 60 min, cumulative dose-response curves were obtained at 30 min intervals for the agonists under study. In some preparations, after achieving stable responses to adrenoceptor agonists, dose-response curves were repeated in the presence of cocaine. Shifts to the left of dose-response curves were always measured as dose-ratios, or log dose-ratios among EC_{50} values. At the beginning of most experiments a dose of tyramine (10^{-5} M) was given to check the release of endogenous catecholamines.

Values of pD_2 were measured as the negative logarithms of EC_{50} to express apparent affinities.

The relative responsiveness ratio (ρ) was measured as previously described (Jurkiewicz *et al.*, 1976), as the relation between the maximal effect of a full agonist (E_m) and the overall maximal effect induced through the effector system of the vas deferens (E_M):

$$\rho = E_m/E_M$$

The value of E_M was arbitrarily taken as the maximal contraction induced by barium chloride (10^{-2}–3×10^{-2} M), in the same experiment in which a full agonist was used. The maximal cumulative contractions for noradrenaline, acetylcholine, 5-hydroxytryptamine (5-HT) or histamine were taken as the respective values of E_m. In general the resting length of the control vas deferens (42.1 ± 1.4 mm, $n = 8$) was reduced by about 40% when the organ was stimulated by a maximal concentration (10^{-2} M) of $BaCl_2$ (length = 24.5 ± 2.1 mm).

Drugs

The following drugs were used: acetylcholine chloride (E. Merck), adrenaline ((−)-epinephrine-D-bitartrate, Sigma); noradrenaline ((−)-arterenol chloride, Sigma); 5-hydroxytryptamine (serotonin, Sigma), histamine (Sigma), tyramine (Schuchardt); barium chloride (J.T. Baker); cocaine hydrochloride (Sigma), and phenoxybenzamine (SKF). Stock solutions of drugs were kept in the freezer and discarded after about 15 days. Working solutions were prepared shortly before each experiment from the stock solution. EDTA (10 µg ml^{-1}) was added to catecholamine solutions to prevent catalytic oxidation by traces of heavy metals. All the chemicals used were ACS certified reagent grade.

Statistics

Significance of differences of contractile and NA release values were analyzed according to an unpaired *t* test (Snedecor & Cochran, 1967).

Results

General characteristics of the vas after hypothermic storage

Drug-induced contractions could be obtained in vas deferens after hypothermic storage, though with a lower amplitude. For instance, cumulative concentration-response curves for barium chloride, obtained after 24, 48, 72 and 96 h showed a progressive decline of the maximal heights, leading to a reduction of about 40% after 72–96 h (Figure 1). However, contrary to the expectations for denervated vasa, spontaneous rhythmic contractions were absent, except for occasional twitches in some preparations. In contrast to barium, the effects for the indirect agonist, tyramine, were reduced to less than 20% after 24 h, and were practically absent after 48 h (Figure 1), indicating a reduction of endogenous noradrenaline. In fact, noradrenaline content was lower than 20% after 72 h, and the activity of DBH had practically disappeared after 48 h (Figure 1). It is noteworthy that after 24 h, when the effects of tyramine had been strikingly reduced, the contents of noradrenaline and DBH were still relatively high, at about 80%, when compared to controls.

The reduction of endogenous NA was corroborated by fluorescence microscopy studies. It was difficult to see a smooth decline of fluorescence at 24 h intervals, due to limitations of our method of measurement and to some individual variation between preparations, but noradrenaline-induced fluorescence was clearly absent after 96 h (not shown).

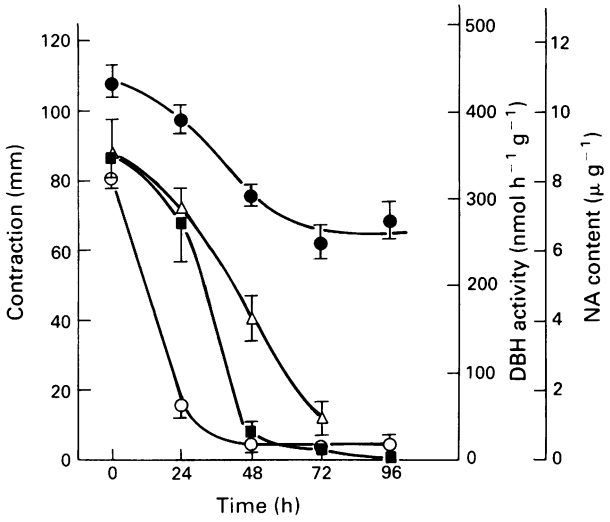

Figure 1 Variation of four pharmacological and biochemical parameters in rat vas deferens, after hypothermic storage: maximal cumulative contraction (under 1 g load and with a 6 fold amplification) for barium chloride (3×10^{-2} M, ●), contraction induced by tyramine (10^{-5} M, ○), concentration of endogenous noradrenaline (NA, △) and dopamine-β-hydroxylase activity (DBH, ■). Parameters were measured at 24 h intervals, as indicated on the abscissae. Each value represents the mean from at least 4 experiments; s.e.mean shown by vertical bars. All values were significantly lower ($P<0.05$) than the respective control (0 h).

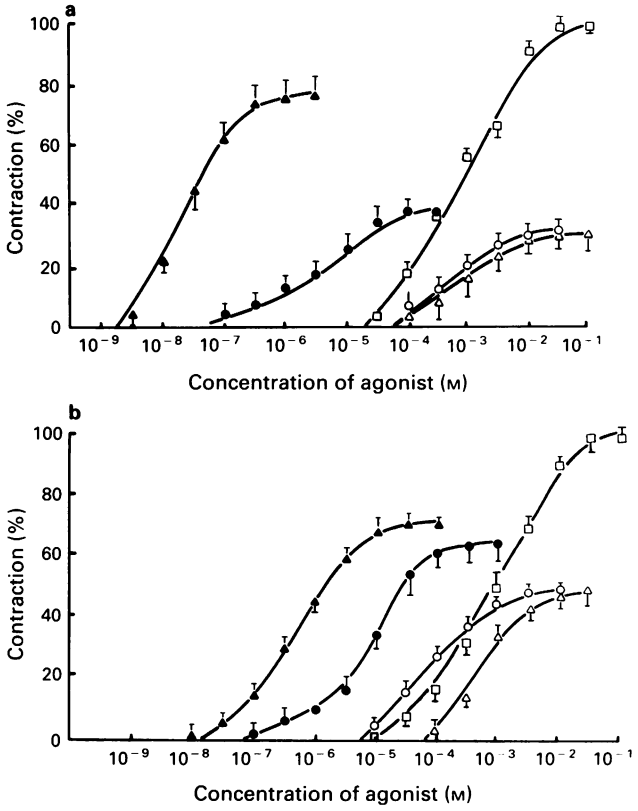

Figure 2 Mean cumulative dose-response curves for noradrenaline (▲), 5-hydroxytryptamine (●), acetylcholine (○), histamine (△) and barium chloride (□) in 72 h cold-stored (a) and contralateral control (b) vas deferens. The effects were plotted as percentages of the respective maximum effect of barium chloride. The 100% contraction was decreased from about 110 mm in controls, to about 63 mm after hypothermic storage. Note that the maximal effect of noradrenaline in relation to barium chloride, was increased after hypothermic storage. Values of pD_2, E_{max} and relative responsiveness (ρ) were measured from these experiments and are shown in Tables 1 and 2. Points are means (± s.e., vertical bars) of at least 5 experiments.

Concentration-response curves

Figure 2 shows that after 72 h, the concentration-response curves for various full agonists, except NA, were reduced after hypothermic storage, when the effects were expressed as percentage values of barium maximal contraction. In addition, a shift to the left was observed for the curve for NA. As a consequence, the value of pD_2 for NA was increased by 1.3 log units, corresponding to a 20 times potentiation. The values of pD_2 for barium chloride, 5-HT and histamine were not significantly changed, while the pD_2 for acetylcholine was slightly reduced (Table 1).

The reduction of maximal effects (Table 2) was not similar for all the agonists studied, ranging between 42 and 66%, when considering absolute contractions (mm). A proportionally larger reduction was observed for acetylcholine, 5-HT and histamine. Because of the partial loss of contractile capacity of the vas deferens after hypothermic storage (Figure 1), these differences in absolute contractions have a limited significance. Therefore, contractions were mostly analysed in relation to the maximal effects of the respective preparations, by using the relative reponsiveness ratio (ρ), as shown below. By means of this ratio, a very small though significant increase, of 10%, was obtained for noradrenaline (Table 2). Concerning the maximal effects of the other three agonists, a reduction was observed for the respective ρ ratios (Table 2), as it occurred for the absolute values.

Neuronal uptake

A reduction of neuronal uptake activity was shown indirectly by measuring two parameters: the reduction of the potentiation induced by cocaine on adrenaline (Ad) concentration-response curves, and the increase of the apparent affinity for the latter agonist. Figure 3 shows a progressive shift to the left of concentration-response curves for Ad, that was proportional to the duration of hypothermic storage. As a consequence, after 72 h the potency of Ad was increased by about 1.5 log units. The same figure shows a decline, from 28 fold to 3 fold, of the ability of cocaine to shift the curves for Ad to the left, after 72 h. As shown in Figure 3a, this decline was simultaneous with the increase of pD_2 values for the latter agonist.

Discussion

Neurochemical, histological and pharmacological parameters were used to show that the rat vas deferens is practically denervated within 72 h of hypothermic storage. In addition, it has been demonstrated that the changes in these parameters follow different time courses, indicating that some properties of the nerve terminals can be more stable than others, during the progression of nerve degeneration. Finally, it was shown that the secondary indicators of denervation, as spontaneous contractions and nonspecific sensitization (Kasuya *et al.*, 1969) could not be detected, contrary to the expectations for the denervated vas deferens.

The decrease of endogenous NA and DBH, the disappearance of fluorescent nerve terminals, and the sharp decline of the contractile activity of tyramine, are strong indications of a degeneration of nerve terminals (Jurkiewicz *et al.*, 1977; 1991). Another characteristic of sympathetic nerves, the ability to take up exogenous catecholamines, has been indirectly shown to be strikingly decreased, through the progressive growth of pD_2 values for adrenaline (Figure 3). The latter values are expected to increase after denervation, since

Table 1 Variation of pD_2 values, expressed as dose-ratios (DR), after hypothermic storage of the vas deferens at 4–6°C for 72 h

Agonist	*pD_2 for control* (A)	*pD_2 after hypothermic storage* (B)	*Log DR* (B-A)	*DR* (antilog (B-A))
Barium chloride	3.0 ± 0.1	3.1 ± 0.1	0.1	1.2
Noradrenaline	6.3 ± 0.1	7.6 ± 0.1*	1.3	20.0
Acetylcholine	4.0 ± 0.1	3.2 ± 0.1*	−0.8	−6.2
Histamine	3.3 ± 0.1	3.3 ± 0.2	0.0	1.0
5-Hydroxytryptamine	5.1 ± 0.1	5.2 ± 0.2	0.1	1.2

All the values are means ± s.e. of at least seven experiments.
*Significantly different from corresponding control value, $P<0.05$.

Table 2 Maximum cumulative effects (E_m) and relative responsiveness ratios (ρ) after hypothermic storage of the vas deferens at 4–6°C for 72 h

Parameter	*Agonist*	*Control* (A)	*After hypothermic storage* (B)	*% Decrease* (A-B)/A × 100
E_m	Barium chloride	108.7 ± 4.7	62.7 ± 4.7*	42
	Noradrenaline	77.6 ± 2.6	41.3 ± 3.7*	47
	Acetylcholine	54.9 ± 4.3	18.9 ± 2.8*	66
	Histamine	50.3 ± 3.1	18.0 ± 2.2*	64
	5-Hydroxytryptamine	67.3 ± 6.0	28.8 ± 4.5*	57
ρ ratio	Noradrenaline	0.69 ± 0.02	0.76 ± 0.03*	10†
	Acetylcholine	0.50 ± 0.02	0.31 ± 0.03*	38
	Histamine	0.48 ± 0.03	0.30 ± 0.03*	37
	5-Hydroxytryptamine	0.63 ± 0.06	0.40 ± 0.06*	36

†A percentage increase was observed in this case.
All the values are means ± s.e. of at least seven experiments.
*Significantly different from corresponding control value, $P<0.05$.

the loss of neuronal uptake permits higher concentrations of exogenous Ad to be available in the vicinity of receptors. This result was corroborated by a parallel decline in the potentiation induced by the neuronal uptake blocker, cocaine (Jurkiewicz & Jurkiewicz, 1976). The increase of pD_2 values or related parameters for NA and Ad, and the loss of cocaine-induced potentiation after inactivation of neuronal uptake, have been exhaustively described in vas, with changes ranging typically between 1.0 and 1.5 log units (Kasuya *et al.*, 1969; Birmingham, 1970; Birmingham *et al.*, 1970; Westfall & Fedan, 1975; Westfall, 1977; Jurkiewicz *et al.*, 1977; 1991). A variation on the time needed for the completion of these changes has also been reported by different authors, ranging from 24 h (Birmingham, 1970) to 4 days, or more (Westfall, 1977; Degaris & Pennefather, 1987).

Although unequal time courses could be seen for different parameters (Figures 1 and 3), most of the values were roughly stabilized within 72 h. The fastest decay was observed for tyramine contractions (Figure 1). It is noteworthy that after nerve regeneration following 5-month transplantation of the vas, the effect of tyramine is the parameter that shows the lowest (practically absent) recovery (Jurkiewicz *et al.*, 1991). Therefore, it can be concluded that the tyramine-induced contraction is the most sensitive indicator of neuronal integrity, in the vas deferens. However, it fails to indicate whether some of the nerve functions, as for instance uptake of catecholamines, are still present.

It is suprising that about 50% of NA was still present after 48 h, since several authors described a loss of at least 90% of NA within the initial 24–48 h after different types of denervation (Kasuya *et al.*, 1969; Lee *et al.*, 1975; Westfall & Fedan, 1975; Westfall *et al.*, 1975). This is probably due to the fact that enzymatic and other types of degradation of NA are diminished during hypothermic storage, thus increasing the stability of NA even if this mediator is freed into the extraneuronal space. As a matter of fact, a similar prolonged presence of NA was observed in guinea-pig taenia caecum after cold storage (Hattori *et al.*, 1972).

An additional unexpected result was the absence of the secondary indicators of denervation, such as spontaneous contractions, and nonspecific potentiation. The latter is expressed as a shift to the left and an increase of the maximal height of concentration-response curves of various unrelated agonists (Kasuya *et al.*, 1969). The shift to the left induced in NA curves cannot be included here because it is a specific phenomenon related to the blockade of neuronal uptake, as discussed. Another specific alteration would be a small shift to the left of acetylcholine curves, due to the loss of cholinesterase (Westfall *et al.*, 1974). However, it has been shown that this enzyme is still active even after 7-day cold storage of the guinea-pig taenia caecum (Hattori *et al.*, 1972), a possibility that can be extended to the vas deferens.

Regarding rhythmic contractions, it is known that the amplitudes attain a maximum after 7 days, but that 30% and 60% of these values are already observed respectively 24 h and 48 h after denervation (Lee *et al.*, 1975). The presence of spontaneous contractions is a nonspecific phenomenon, since it can also be observed in the vas deferens of castrated rats (Martins & Valle, 1939). It can also be seen in the vas deferens from 30 to 90 min after injecting 6-hydroxydopamine, probably because of the release of NA (Furness *et al.*, 1970).

In relation to nonspecific sensitization, the only alteration observed here was a trivial increase of 10% on ρ value for NA receptors. This contrasts with the high rises described for the height of concentration-response curves for NA (from about 30% to 400%) and, to a lesser degree, for acetylcholine (Kasuya *et al.*, 1969; Kasuya & Suzuki, 1978; Westfall *et al.*, 1972; 1974; Goto *et al.*, 1976; 1979; Lee *et al.*, 1975; Murdock *et al.*, 1977). Increases were also observed for 5-HT (Goto *et al.*, 1976) and for histamine in guinea-pig vas (Westfall *et al.*, 1972), but not for potassium (Lee *et al.*, 1975). An increased maximal effect can either indicate an increase in the density of receptors or a change in the chain

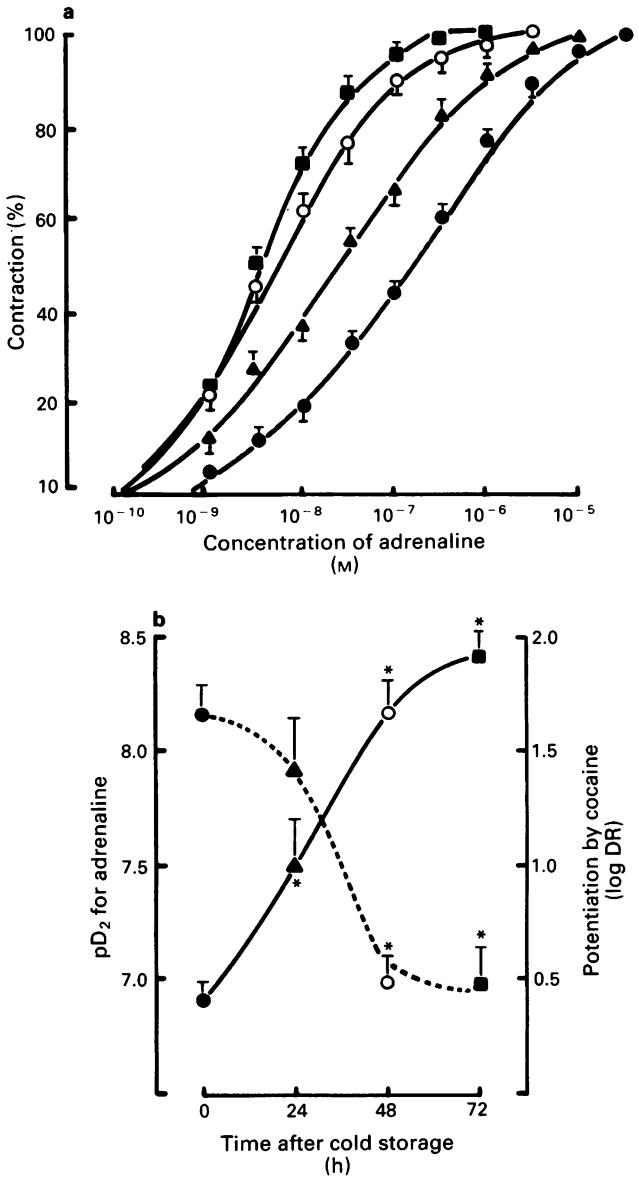

Figure 3 Concentration-response curves (a) for adrenaline in controls (●) or after hypothermic storage for 24 h (▲), 48 h (○) or 72 h (■). In (b) are shown the increase of pD_2 values for adrenaline, measured from the respective curves (complete line), as well as the decay of the potentiation induced in the respective curves by 10^{-5} M cocaine (dotted line). This potentiation was expressed as the logarithm of a dose ratio (log DR) for adrenaline, representing the leftward shift induced by cocaine on adrenaline curves. Points are means (± s.e., vertical bars) of at least 5 experiments. $^*P<0.05$ compared with controls.

of events leading from drug-receptor interaction to the effect. A theoretical analysis of the meaning and limitations of the changes of maximal effects, measured through the ρ ratio, has been presented previously (Jurkiewicz *et al.*, 1976). A few situations have been described in which a nonspecific potentiation was not observed: DeGaris & Pennefather (1987) could not detect increases in maximal effects for adrenergic and cholinergic agents by denervating only the epididymal part of the vas deferens. Kasuya & Suzuki (1978) have shown that the effect of ACh is practically abolished in the prostatic part of the vas, after denervation. In addition, Sannomiya & DeMoraes (1979), using the denervated guinea-pig vas, could not show an increased maximal effect for NA when recording contractions *in situ*, contrary to experiments *in vitro*. The results of Kasuya & Suzuki (1978) are compatible with our finding that the effects of acetylcholine are reduced (Tables 1 and 2). The reasons for this specific reduction remain to be explained.

The presence of nonspecific changes, such as spontaneous movements and sensitization, is probably due to complex mechanisms. It was previously shown that the primary changes represented by nerve degeneration are followed by secondary changes on smooth muscle tissue, that can be represented: (a) morphologically, by an increase of the number of cell-to-cell close junctions, or nexuses, that are twice as many as in controls (Westfall *et al.*, 1975); (b) electrophysiologically, by a growth of electrical coupling between cells and improved synchronization during drug-induced contractions (Lee *et al.*, 1975; Goto *et al.*, 1976); (c) histochemically, by an increase of non-neuronal ATP from the 2nd day on (Westfall *et al.*, 1975) and of endogenous peptides, such as vasoactive intestinal peptide and calcitonin gene-related peptide (Aberdeen *et al.*, 1992). The discussion of these points, as well as of the reinnervation that commences after about 4 days and takes several months to be completed (Burnstock, 1981; Jurkiewicz *et al.*, 1991), is out of the scope of the present publication.

A fall of the maximal contractile ability of the preparation (Figure 2 and Table 1) shows that smooth muscle cells were also injured during hypothermic storage denervation. When the vas undergoes hypothermic storage, the secondary mechanisms stated above are probably blocked, because of the lack of irrigation and nutrition, added to hypoxia and absence of the trophic influence of testosterone. However, one could argue that this damage is not simply due to the cooling process, since storage at room temperature would lead to a faster degeneration and loss of function. In this way cooling can be seen as a tool to slow down the degeneration process. On the other hand, since cold storage causes some changes in ionic balance in smooth muscle (Fukuda & Shibata, 1972), it is still unknown whether these electrolyte variations do influence drug-induced responses in vas.

It is concluded that hypothermic storage of the vas deferens differs from other proposed methods for denervation in at least two properties: (a) the primary pharmacological signs of nerve degeneration are not followed by the secondary indicators of denervation, such as spontaneous contractions and nonspecific sensitization; (b) from a methodological standpoint, this procedure seems to be easier to perform than *in vivo* denervations, since it avoids the postoperative maintenance of the rats, and a second surgical procedure for the removal of the vas.

References

ABERDEEN, J., MILNER, P., LINCOLN, J. & BURNSTOCK, G. (1992). Guanethidine sympathectomy of mature rats leads to increases in calcitonin gene-related peptide and vasoactive intestinal polypeptide-containing nerves. *Neuroscience*, **47**, 453–461.

ANTON, A.H. & SAYRE, D.F. (1962). A study of the factors affecting the aluminum oxide-trihydroxindole procedure for the analysis of catecholamines. *J. Pharmacol. Exp. Ther.*, **138**, 360–375.

BIRMINGHAM, A.T. (1970). Sympathetic denervation of the smooth muscle of the vas deferens. *J. Physiol.*, **206**, 645–661.

BIRMINGHAM, A.T., PATTERSON, G. & WOJCICKI, J. (1970). A comparison of the sensitivities of innervated and denervated rat vasa deferentia to agonist drugs. *Br. J. Pharmacol.*, **39**, 748–754.

BURNSTOCK, G. (1981). Development of smooth muscle and its innervation. In *Smooth Muscle: an Assessment of Current Knowledge*. ed. Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 431–457. London: Edward Arnold.

DEGARIS, R.M. & PENNEFATHER, J.N. (1987). Prolonged supersensitivity to noradrenaline of smooth muscle of the epididymal half of the rat vas deferens denervated by vasectomy. *J. Auton. Pharmacol.*, **7**, 267–279.

FALCK, B. (1962). Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. *Acta Physiol. Scand.*, **56**, 2–25.

FESTING, M.F.W. (1980). *International Index of Laboratory Animals.* UK: Medical Research Council.

FUKUDA, H. & SHIBATA, S. (1972). The effect of cold storage on the inhibitory action of isoprenaline, phenylephrine and nicotine on the mechanical and membranal activities of guinea-pig taenia caecum. *Br. J. Pharmacol.*, **46**, 438–448.

FURNESS, J.B., CAMPBELL, G.R., GILLARD, S.M., MALMFORS, T., COBB, J.L.S. & BURNSTOCK, G. (1970). Cellular studies of sympathetic denervation produced by 6-hydroxydopamine in the vas deferens. *J. Pharmacol. Exp. Ther.*, **174**, 111–122.

GOTO, K., MASAKI, T., SAITO, A. & KASUYA, Y. (1979). Denervation-like supersensitivity of the rat vas deferens induced by local application of colchicine to the hypogastric plexus. *J. Pharmacol. Exp. Ther.*, **209**, 376–381.

GOTO, K., MASUDA, Y. & KASUYA, Y. (1976). The effect of denervation on the synchronization of contraction of the rat vas deferens. *Eur. J. Pharmacol.*, **36**, 395–404.

HATTORI, K., KURAHASAHI, K., MORI, J. & SHIBATA, S. (1972). The effect of cold storage on the adrenergic mechanisms of intestinal smooth muscle. *Br. J. Pharmacol.*, **46**, 423–437.

JURKIEWICZ, A., ABDO, A.O., JURKIEWICZ, N.H., GUEDES, A.O. & SOUCCAR, C. (1976). Relative responsiveness (ρ): a critical analysis of a new method in receptor differentiation. *Gen. Pharmacol.*, **7**, 93–101.

JURKIEWICZ, N.H. & JURKIEWICZ, A. (1976). Dual effect of α-adrenoceptor antagonists in rat isolated vas deferens. *Br. J. Pharmacol.*, **56**, 169–178.

JURKIEWICZ, N.H., JURKIEWICZ, A. & GARCIA, A.G. (1991). Reinnervation of the transplanted vas deferens: differential recovery of various biochemical and pharmacological parameters. *J. Neural. Transm.*, **85**, 83–94.

JURKIEWICZ, N.H., JURKIEWICZ, A., GOMES, C.B. & AUCELIO, J.G. (1977). Pharmacodynamic analysis of the rat isolated vas deferens after transplantation to intestinal wall. *J. Pharmacol. Exp. Ther.*, **203**, 112–119.

KASUYA, Y., GOTO, K., HASIMOTO, H., WATANABE, H., MUNUKATA, H. & WATANABE, M. (1969). Nonspecific denervation supersensitivity in the rat vas deferens 'in vitro'. *Eur. J. Pharmacol.*, **8**, 177–184.

KASUYA, Y. & SUZUKI, N. (1978). Regional differences in the effects of denervation, cocaine and chronic reserpine administration on the respones of the rat vas deferens to norepinephrine and acetylcholine. *Arch. Int. Pharmacodyn.*, **236**, 202–213.

KLEIN, R.L. (1982). Chemical composition of the large noradrenergic vesicles. In *Neurotransmitter Vesicles.* ed. Klein, R.L., Lagercrants, H. & Zimmermann, H. pp. 133–174. New York: Academic Press.

LEE, T.J.-F., WESTFALL, D.P. & FLEMING, W.W. (1975). The correlation between spontaneous contractions and postjunctional supersensitivity of the smooth muscle of the rat vas deferens. *J. Pharmacol. Exp. Ther.*, **192**, 136–148.

MARTINS, T. & VALLE, J.R. (1939). Endocrine control of the motility of the male accessory genital organs. *Endocrinology*, **25**, 80–90.

MOLINOFF, P.B., WEINSHILBOUM, R. & AXELROD, J. (1971). A sensitive enzymatic assay for dopamine β-hydroxylase. *J. Pharmacol. Exp. Ther.*, **178**, 425–431.

MURDOCK, S.D., EVANS, B.K., HEATH, J.W., HILL, C.E. & BURNSTOCK, G. (1977). Effects of chronic bretylium treatment on the sympathetic neuron and the smooth muscle of the rat. *Eur. J. Pharmacol.*, **43**, 225–235.

SANNOMIYA, P. & DE MORAES, S. (1979). Denervation supersensitivity to noradrenaline in the guinea-pig vas deferens in vivo: absence of the postjunctional component. *Eur. J. Pharmacol.*, **54**, 167–171.

SNEDECOR, G.W. & COCHRAN, G.C. (1967). In *Statistical Methods.* (6th ed). Ames, Iowa, USA: Iowa State University Press.

WAKADE, A.R. (1978). Time course of degeneration of short and long postganglionic sympathetic nerve fibres and effect of pentobarbitone and colchicine on degeneration. *Br. J. Pharmacol.*, **62**, 553–561.

WAKADE, A.R. (1979). Recent developments in degeneration of the sympathetic neuron. *Gen. Pharmacol.*, **10**, 351– 357.

WESTFALL, D.P. (1977). The effects of denervation, cocaine, 6-hydroxydopamine and reserpine on the characteristics of drug-induced contractions of the depolarized smooth muscle of the rat and guinea-pig vas deferens. *J. Pharmacol. Exp. Ther.*, **201**, 267–275.

WESTFALL, D.P. & FEDAN, J.S. (1975). The effect of pretreatment with 6-hydroxydopamine on the norepinephrine concentration and sensitivity of the rat vas deferens. *Eur. J. Pharmacol.*, **33**, 413–417.

WESTFALL, D.P., LEE, T.J.-F. & STITZEL, E. (1975). Morphological and biochemical changes in supersensitive smooth muscle. *Fed. Proc.*, **34**, 1985–1989.

WESTFALL, D.P., MCPHILLIPS, J.J. & FOLEY, D.J. (1974). Inhibition of cholinesterase activity after postganglionic denervation of the rat vas deferens: evidence for prejunctional supersensitivity to acetylcholine. *J. Pharmacol. Exp. Ther.*, **189**, 493–498.

WESTFALL, D.P., MCCLURE, D.C. & FLEMING, W.W. (1972). The effects of denervation, decentralization and cocaine on the response of the smooth muscle of the guinea-pig vas deferens to various drugs. *J. Pharmacol. Exp. Ther.*, **181**, 328–338.

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