Release of noradrenaline and dopamine by nerve stimulation in the guinea-pig and rat vas deferens

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1 Spontaneous and nerve stimulated release of noradrenaline and dopamine from rat and guinea-pig vas deferens have been measured electrochemically after separation by high performance liquid chromatography (h.p.l.c.).

2 In the absence of nerve stimulation both noradrenaline (NA) and dopamine were released into the bathing fluid in the rat but in the guinea-pig only noradrenaline could be detected. Drugs which block neuronal and extraneuronal uptake of catecholamines had little effect on spontaneous overflow but both tetraethylammonium and phenoxybenzamine increased overflow.

3 Transmural nerve stimulation (5–10 Hz) increased catecholamine overflow in both species and dopamine release was now measurable from the guinea-pig vas. In the rat, the proportion of dopamine to NA was unchanged from that released spontaneously. The release of both amines was little affected by drugs that block neuronal and extraneuronal uptake and a monoamine oxidase inhibitor, but was inhibited by tetrodotoxin $0.2\,\mu g\,ml^{-1}$.

4 In the guinea-pig tetraethylammonium 10 mM doubled overflow and phenoxybenzamine 10^{-5} M increased it by five times but the dopamine percentage remained constant and equal to the control.

5 Following nerve stimulation the amount of dopamine released expressed as a percentage of total catecholamine release was 6% for the rat and 1.3% for the guinea-pig. These values were considerably higher than the comparable figures for dopamine: NA content of the two tissues (2% and 0.5% respectively). Repeated periods of stimulation depleted these tissue stores and the depletion of dopamine was significantly greater than that of NA.

6 Our interpretation of these results is that both dopamine and NA are released from a common store during normal noradrenergic transmission. While all or most of the axonal dopamine is contained in this releasable pool, most of the axonal NA lies in a second, less readily released pool.

Introduction

Most adrenergically innervated tissues contain both noradrenaline (NA) and dopamine, the proportion varying with species and tissue (Bell & Gillespie, 1981). The localisation and function of this dopamine is obscure. Manoeuvres known to increase activity in peripheral sympathetic nerves increase the plasma levels of dopamine as well as NA and adrenaline (Callingham & Barrand, 1976; Christensen *et al.*, 1976; Kvetnansky *et al.*, 1978). Both radiolabelled dopamine and NA taken up by the cat spleen can subsequently be released by splenic nerve stimulation (Musacchio *et al.*, 1966). These results

suggest that dopamine in sympathetic nerves, presumably as a NA precursor, can be released by nerve stimulation. Another possibility is that the dopamine is derived from true peripheral dopaminergic nerves (Bell, 1982) or, alternatively, from sources other than terminal axons, such as chromaffin cells or the small intensely fluorescent cells (SIF cells) that are often associated with peripheral autonomic ganglia (Libet, 1979).

We have investigated this problem in the isolated saline-bathed vas from the rat and guinea-pig, species with a very different proportion of dopamine in their tissue stores and in which the biochemical data on tissue stores of catecholamines indicates the innervation is purely noradrenergic (Bell & Gillespie, 1981). We first looked to see whether transmural nerve stimulation can liberate both NA and

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dopamine and whether the proportion of the two amines in the overflow reflects the difference in the tissue stores in the two species. Secondly, we have examined the effects of drugs known to increase the release of NA to see whether there was a corresponding increase in dopamine release. The results show that dopamine is released by nerve stimulation together with NA and suggest that both amines are stored in a common vesicular pool. However, while this pool constitutes the bulk of the axonal dopamine, it represents only a part of the total NA store.

Methods

Adult guinea-pigs and Wistar rats were stunned and bled and the vasa deferentia removed. Paired tissues were stripped of their mesentery and mounted vertically in a 2.0 ml Perspex bath through which oxygenated Krebs solution could be pumped from below to be removed at the upper surface by suction through a steel cannula in a side channel. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and dextrose 11 and was bubbled with 95% O_2 plus 5% CO_2 both in the reservoir and within the organ bath. Ascorbic acid $20 \,\mu g \, ml^{-1}$ and EDTA $10 \,\mu g \, ml^{-1}$ were added to the Krebs solution to prevent oxidative destruction of catecholamines.

Intramural nerves were stimulated by passing 1 ms pulses of supramaximal voltage from a Grass S88 stimulator between a fine silver wire threaded the whole length of the lumen of the tissue and an open coil of the same wire surrounding the tissue. Trains of either 1,800 (rat) or 2,700 (guinea-pig) pulses were used, separated by rest periods of 30 min. The frequencies used are indicated in the text.

When the nerves were stimulated, the flow of saline was stopped for the period of nerve stimulation plus 1.5 min. For the guinea-pig vas deferens with 2,700 pulses at 10 Hz, this gave a total stop flow period of 6 min. In the rat vas deferens with a shorter train of 1,800 stimuli, a similar 6 min stop flow collection period was used even though this meant included a longer post-stimulation period. In the rat, stimulated at 5 Hz, the collection period was extended to 7.5 min. At the end of the stop flow period the entire bath volume was aspirated, the pH of the sample immediately adjusted to pH 8.6 by adding 0.5 ml of 0.5 M Tris buffer at this pH, and the alkaline mixture shaken for 10 min with 50 mg of acid washed alumina to absorb the catecholamines. At the end of this time the buffer was aspirated and the alumina washed with a weak 5 mM Tris buffer at pH 8.6 which was in its turn aspirated. The absorbed catecholamines were then eluted from the alumina with $100-300 \,\mu l$ of 0.1 N perchloric acid containing 4×10^{-4} M sodium

metabisulphite. This total volume was injected into the high performance liquid chromatography (h.p.l.c.) electrochemical detector system and the separate catecholamines measured.

Tissue catecholamines and the effect of nerve stimulation on this were measured in pairs of vasa. From each pair the left was stored on ice while the right was set up for transmural stimulation. After three periods of stimulation at 30 min intervals, the right vas was removed from the chamber and it, and the stored left vas, were separately chopped finely with scissors and then homogenized in a motor-driven teflon pestle in glass homogenizer with 2 ml of ice-cold 0.1 N perchloric acid containing 4 mg $100 \, ml^{-1}$ of sodium metabisulphite. Homogenization was carried out at 4°C with two periods of 30 s drive to the teflon pestle at full power. The homogenates were centrifuged at 4°C and 3,000 g for 15 min and the supernatant decanted. The catecholamines in 0.5 ml aliquots of supernatant were absorbed on 50 mg of acid-washed alumina and subsequently treated like samples of bath fluid.

The h.p.l.c. system was modified during the project. Originally a 50 cm column of i.d. 0.25 cm packed with pellicular Corasil CX cation exchange resin was used to separate the amines and a home-made electrochemical cell with a carbon paste electrode to detect them. The column was replaced with a 15 cm Hypersil C18 reverse phase column, i.d. 5 mm and the carbon paste electrode with a glassy carbon electrode. With ion pairing the reverse phase column gave better separation of the amines and the glassy carbon electrode a more stable base line with only a little loss of sensitivity. The electrochemical detector was run at $+0.55$ V and the mobile phase for ion exchange chromatography was 63 mM citrate-acetate buffer pH 5.2 and for ion-pair chromatography 100 mM phosphate buffer pH 3.2 and containing the appropriate pairing reagent sodium octyl sulphonate 0.2 mM plus 10% methanol. The limit for measurement of NA with this system was 100 pg.

As well as measuring the nerve-stimulated release of catecholamines, the spontaneous overflow in a similar 6 min of stop flow, but without nerve stimulation, was also measured. This value, corrected for recovery, was subtracted from the nerve-stimulated overflow similarly corrected to give the extra overflow as a result of nerve stimulation. Finally, at the end of each experiment, 10 ng of NA and 10 ng of dopamine were added to the bath in the presence of tissue, left for 6 min of stop flow then removed and the recovery of the two amines measured. The calculation of NA recovery was complicated by the measurable spontaneous release of that amine. In calculating the actual recovery from 6 min exposure to tissue and subsequent extraction, the recovery of dopamine which was not subject to this complication

was used. Dopamine recoveries averaged 38% and overflows have been corrected for this. The recovery of the two amines in the absence of tissue was equal and in all experiments over 70%.

Drugs

Drugs were: L-ascorbic acid (BDH), desmethylimipramine hydrochloride (Geigy), and dopamine hydrochloride (Koch-Light), ethylenediaminetetraacetic acid disodium salt (EDTA, Sigma), (−)-noradrenaline bitartrate (Koch-Light), 17β-oestradiol (Sigma), phenoxybenzamine hydrochloride (SKF), tetraethylammonium bromide (Koch-Light), tetrodotoxin (TTX, Sigma) and tranylcypromine sulphate (SKF).

Results

Spontaneous and nerve-stimulated overflow from the rat vas

The first experiments were performed on the rat vas since the proportion of dopamine in the total catecholamine tissue stores is greater in this species than in the guinea-pig. In six experiments transmural

Table 1 Spontaneous and nerve-stimulated overflow of noradrenaline (NA) and dopamine (DA) in pg from paired rat vas deferens

	n	First stimulation	Rest (Spontaneous)	Second stimulation
Cocaine 10^{-5} M during 2nd stimulation period				
NA	6	1343 ± 317	—	1783 ± 442
DA		130 ± 88	—	147 ± 35
DA/NA		9.7%	—	8.2%
Cocaine 10^{-5} M present throughout				
NA	11	2524 ± 460	1234 ± 306	1736 ± 380
DA		144 ± 25	84 ± 14	137 ± 22
DA/NA		5.7%	6.8%	7.9%
Pbz 10^{-5} M + cocaine 10^{-5} M during 2nd stimulation period				
NA	5	2760 ± 621	—	9700 ± 1616
DA		126 ± 56	—	260 ± 63
DA/NA		4.7%	—	2.7%

The first set of six experiments are from tissues treated with cocaine (10^{-5} M) between the first and second periods of stimulation; the second set of eleven experiments are from tissues treated with cocaine (10^{-5} M), before the first stimulation period and the last set of five experiments the effect of adding phenoxybenzamine (Pbz 10^{-5} M) together with cocaine between the first and second stimulation periods. Transmural stimulation was by 1,800 pulses at 5 Hz.

stimulation released both NA and dopamine into the bathing fluid. Cocaine 10^{-5} M added to block neuronal reuptake increased slightly the amounts of both amines but these increases were not statistically significant. However, in all subsequent experiments cocaine was added to the bathing fluid. The second group of eleven experiments in Table 1 shows a comparison between spontaneous release in the absence of nerve stimulation and that caused by transmural electrical stimulation. Both NA and dopamine were released into the bathing fluid without nerve stimulation. The amounts were greatest immediately after setting up the preparations and declined with time to a lower stable value. In these circumstances transmural stimulation with 1,800 pulses at 5 Hz doubled the release of both amines. The proportion of dopamine to NA which averaged about 6.8% remained constant with no obvious difference between nerve stimulated and spontaneous release.

In another five experiments, phenoxybenzamine 10^{-5} M, in addition to cocaine, was added to the bathing solution. Phenoxybenzamine increased the overflow of both amines following nerve stimulation and the increase in NA (three to five fold) was greater than that of dopamine (two fold) Table 1.

Spontaneous overflow in the guinea-pig vas

In stop flow samples without nerve stimulation, NA, but not dopamine, could always be detected in the bathing fluid. If the stop flow sample was taken soon after setting-up the preparation, values as high as 50 ng were reached in a 6 min period. These values fell rapidly to a stable figure of 3.0 ± 0.6 ng total or 1.4 ng ml^{-1}. The addition of the drugs used to block uptake or increase catecholamine release by nerve

Table 2 Spontaneous overflow of noradrenaline (NA) from the guinea-pig vas deferens

Sample	n	Overflow 6 min^{-1} (ng)	Rate of overflow (pg s^{-1})
Control	22	3.0 ± 0.6	8.4 ± 1.7
DMI + 17β + Tran	8	4.4 ± 0.8	12.2 ± 2.1
TEA + DMI	8	10.7 ± 1.9*	29.8 ± 5.3*
Pbz	4	13.3 ± 4.7*	36.9 ± 13.1*
TEA + DMI + Pbz	5	4.4 ± 1.0	12.4 ± 2.9

The results are expressed as ng per 6 min collection period and also as a rate of overflow in pg s^{-1} and the table shows the effect of desmethylimipramine (DMI) plus 17β-oestradiol (17β) and tranylcypromine (Tran) all at 10^{-5} M, of phenoxybenzamine (Pbz, 10^{-5} M) and of tetraethylammonium (TEA, 10 mM). Each value is the mean ± s.e. and n = number of experiments. Values significantly different from the control by Student's t test are indicated by asterisks: *$P < 0.001$.

stimulation also influenced spontaneous release as Table 2 shows. In this Table both the absolute accumulation of NA and the rate of accumulation necessary to reach these values are given. Uptake blockers plus the monoamine oxidase inhibitor tranylcypromine (all 10^{-5} M) increased the spontaneous overflow but this increase was not statistically significant. In contrast both tetraethylammonium (TEA, 10 mM) in the presence of desmethylimipramine (DMI) and phenoxybenzamine (Pbz, 10^{-5} M) alone increased the spontaneous overflow by 3.5 and 4.5 times respectively and these increases were significant. Surprisingly, the combination of TEA, DMI and Pbz far from having an additive effect seemed to cancel one another out so that the spontaneous release was little different from that with uptake blockers alone and, like that value, not significantly different from control.

Nerve-stimulation overflow in the guinea-pig vas

The experiments with the rat vas showed both NA and dopamine were liberated by nerve stimulation. We wished to compare these results with the guinea-pig which has a lower fractional content of dopamine in the tissue and likely, therefore, to release a smaller fraction. For this reason and because the measurement of dopamine released from the rat vas was already difficult, we increased the number of stimuli to 2,700 and where dopamine was to be measured more exactly we combined samples as described later.

Transmural stimulation with trains of 2,700 pulses

in the presence of uptake blocking drugs plus tranylcypromine, increased the overflow of NA some 11 times from 3.0 ± 0.6 ng in 6 min to over 33 ng in 6 min and, in these circumstances, dopamine was also detected but in amounts insufficient for accurate measurement. We, therefore, concentrated first on factors influencing the release of NA. Table 3 shows the effect of stimulation frequency, of train length and of train repetition on the release of NA. With long trains of 2,700 pulses at 10 Hz, large amounts of NA appeared in the bathing fluid but these progressively declined in each period of stimulation so that in the third the overflow was only 65% of the first. When shorter trains of 200 pulses at this same frequency were used there was no decline. Furthermore, though the total NA was greater with the long trains the average rate of overflow was actually less than with the short trains, suggesting a decline in transmitter release towards the end of the longer trains. Advantage was taken of this ability to maintain a constant overflow in successive stimulation periods with short trains of 200 pulses to examine the effects of frequency. Four short periods of stimulation at 2, 5, 10 and 15 Hz were applied in each of six experiments and the average release at each frequency calculated. The results are shown in Table 3. At 2 Hz and 5 Hz the total recovery of NA was similar at just over 4 ng and a rate of release of 46 and 105 pg s^{-1}. At 10 Hz and 15 Hz the total NA fell progressively but this was compensated by the shorter duration of nerve stimulation so that the rate of release rose to a plateau of just under 200 pg s^{-1} at 10 Hz and remained constant at this level.

Table 3 The effect of frequency, of train length and of train repetition on the total overflow and the rate of overflow of noradrenaline (NA) as a result of transmural stimulation in the guinea-pig vas deferens

| Conditions | n | Total NA overflow (ng) in each period of stimulation | | | |
		First	Second	Third	Fourth
2700 at 10 Hz	8	34 ± 4.6	27.8 ± 4.9	23.3 ± 3.0	—
200 at 10 Hz	11	4.6 ± 0.3	4.5 ± 0.5	4.6 ± 0.7*	4.6 ± 0.12*
200 at 2 Hz	6	4.6 ± 0.7	—	—	—
200 at 6 Hz		—	4.2 ± 0.8	—	—
200 at 10 Hz		—	—	3.6 ± 0.7	—
200 at 15 Hz		—	—	—	2.6 ± 0.6
		Rate of NA overflow (pg s^{-1})			
2700 at 10 Hz	8	122.3 ± 20	102 ± 18	86.5 ± 11	—
200 at 10 Hz	11	231 ± 15	226 ± 23	230 ± 33	230 ± 62
200 at 2 Hz	6	46 ± 7	—	—	—
200 at 5 Hz		—	105 ± 20	—	—
200 at 10 Hz		—	—	180 ± 33	—
200 at 15 Hz		—	—	—	195 ± 43

All experiments were done in the presence of desmethylimipramine, 17β-oestradiol and tranylcypromine, all in a concentration of 10^{-5} M. Each value is the mean ± s.e. and n = number of experiments. The values marked with an asterisk are from nine and three experiments respectively.

Table 4 Nerve stimulated overflow of noradrenaline (NA) from the guinea-pig vas showing the effect of the combined uptake blocking drugs desmethylimipramine (DMI, 10^{-5}M) and 17β-oestradiol (17β, 10^{-5}M) with the monoamine oxidase inhibitor tranylcypromine (Tran, 10^{-5}M) of tetraethylammonium (TEA, 10^{-5}M) and of phenoxybenzamine (Pbz, 10^{-5}M)

Conditions	n	Total NA overflow (ng) Period of Stimulation		
		First	Second	Third
Control	8	32.9±3.4	27.1±3.4	22.1±3.2
		Drug present		
DMI + 17β + Tran	1	20	27	19
TEA + DMI	1	31	63	54
Pbz	1	23	109	102

The nerves were stimulated by trains of 2700 stimuli at 10 Hz. Where drugs were tested they were present during the second and third periods of stimulation. The control results are the averages of eight experiments.

Effect of drugs on overflow

The effects of uptake blocking drugs, of TEA and of Pbz were separately examined on the nerve stimulated overflow of noradrenaline and the results are shown in Table 4. These experiments were intended to define which drug or drug combination gave the biggest increase in transmitter overflow as a preliminary to measuring their effects on dopamine release. For this reason single experiments were performed. As Table 4 shows, the combined uptake blockers and monoamine oxidase inhibitor produced only a small increase in noradrenaline overflow. In these experiments DMI was used but the results were similar to those with cocaine in the rat vas. TEA combined with DMI approximately doubled the overflow and Pbz increased overflow by about 5 times. These two drugs, TEA and Pbz, were, therefore, used to examine their effect on the overflow of dopamine as well as NA. Since Pbz itself blocks the neuronal uptake process for catecholamines, TEA was combined with DMI.

The effects of drugs on the nerve-stimulated overflow of noradrenaline and dopamine

To measure dopamine accurately, several bath samples were combined. In the first set of experiments, to demonstrate that dopamine release was blocked by

TTX, six pairs of vasa from six animals were used. Each pair was stimulated with 2,700 pulses at 10 Hz for three periods at 30 min intervals. After the first stimulation period, TTX 0.2 μg ml^{-1} was added to the Krebs and the remaining two periods of stimulation performed in the presence of this drug. All of the first periods of stimulation from the six tissues were combined to form a 'control' sample. All of the second and third periods of stimulation from the six tissues were combined to form a 'TTX sample'. As Table 5 shows, the control sample contained 320 ng of NA and 2.3 ng of dopamine, whereas the larger TTX sample contained only 6.1 ng NA and dopamine was undetectable (less than 200 pg). The release of both amines, therefore, is by a TTX-sensitive process, presumably by conducted nerve action potentials.

The effect of TEA, Pbz and their combination was then examined. In these experiments, because the drugs increased release, it was possible to use fewer samples and still measure dopamine accurately. A different experimental arrangement was, therefore, used. In each experiment two pairs of vas deferens were used each stimulated for three periods with 2,700 pulses at 10 Hz and the six samples then combined for a single extraction and assay. Groups of five or six such experiments were then done in the absence of drugs, and in the presence of each drug or drug combination. The results are shown in Table 6. In the absence of drugs the average overflow of NA was 260 ng and for dopamine 3.3 ng, giving a dopamine:NA ratio of 1.3%. TEA increased the overflow of both amines two fold but with a little changed dopamine:NA ratio of 1.8%. Pbz increased the overflow by just over four times but still the dopamine:NA ratio remained almost the same as under control conditions. Curiously, just as with spontaneous overflow, the combination of TEA and Pbz gave no additive effect. If anything, overflow was less than with Pbz alone. The dopamine:NA ratio, however, remained constant at 1.3%. The second

Table 5 The effect of tetrodotoxin (TTX) 0.2 μg ml^{-1} on the overflow of noradrenaline (NA) and dopamine (DA) from the guinea-pig vas deferens in response to transmural stimulation with 2700 pulses at 10 Hz

Control (ng)			TTX (ng)		
NA	DA	DA/NA%	NA	DA	DA/NA%
320	2.3	0.73	6.1	0	—

Six preparations were used and the first period of stimulation from each (six samples) were combined to give the control sample. The second and third periods of stimulation in the presence of TTX were combined (twelve samples) as the TTX sample.

Table 6   The effect of tetraethylammonium (TEA, 10 mM), in the presence of desmethylimipramine (DMI, 10^{-5} M), of phenoxybenzamine (Pbz, 10^{-5} M) and of the combination of these drugs on the overflow of noradrenaline (NA) and dopamine (DA) from the guinea-pig vas deferens in response to transmural stimulation with 2700 pulses at 10 Hz

	n	NA	DA	DA/NA%
		Total overflow (ng)		
Control	5	260 ± 10	3.3 ± 0.45	1.3 ± 0.1
TEA + DMI	5	467 ± 40**	8.8 ± 2.0*	1.8 ± 0.3
Pbz	5	1163 ± 162**	15.7 ± 2.6**	1.4 ± 0.16
TEA + DMI + Pbz	6	984 ± 42***	13.2 ± 2.0**	1.3 ± 0.15
		Rate of overflow ($pg\,s^{-1}$)		
Control	5	160 ± 6	2.0 ± 0.3	—
TEA + DMI	5	289 ± 25	5.4 ± 1.3	—
Pbz	5	718 ± 112	9.7 ± 1.8	—
TEA + DMI + Pbz	6	608 ± 28	8.1 ± 1.4	—

Each individual observation is derived from a sample in which the bath fluid from three successive stimulation periods from two pairs of tissues was combined (total of six periods), n = number of such observations and the values in the table are the mean $\pm$ s.e. of these. Asterisks indicate values statistically different from control at the 0.001, 0.01 or 0.05 level.

half of Table 6 expresses these values as rates of overflow. The control values, not surprisingly, are a little less than those in Table 3, since they are the average of three periods of stimulation in each preparation during which overflow steadily declined (Table 3). In the presence of TEA and, especially, of Pbz, the rate of NA release rises to values much higher than were ever observed in controls.

The effects of nerve stimulation on tissue amine stores

The results in the preceding paragraph show that drugs may greatly increase transmitter release but the percentage of dopamine remains constant and close to the 1.3% value for control. This value is higher than the reported fraction of 0.5% in the tissue stores (Bell & Gillespie, 1981). Repeated periods of stimulation with 2,700 pulses at 10 Hz results in a steady decline in NA overflow and even within a single period of stimulation the average rate of release is only half that with short trains suggesting a decline in transmitter release within the train. These results are consistent with depletion of the stores available to the nerve impulse. If this is so, then the higher percentage of dopamine in the overflow might cause a greater depletion of dopamine. We have compared the levels of NA and dopamine in unstimulated vasa with those stimulated for three periods with 2,700 pulses at 10 Hz. In the unstimulated vas the NA content averages $18.9 \pm 1.4\,\mu g\,g^{-1}$ $(n = 6)$ and dopamine $0.15 \pm 0.03\,\mu g\,g^{-1}$ $(n = 6)$ giving a dopamine:NA ratio of $0.82 \pm 0.1\%$ $(n = 6)$. After repeated stimulation there was a significant depletion of both amines, NA to $13.5 \pm 1.1\,\mu g\,g^{-1}$ $(n = 6)$ and dopamine to $0.07 \pm 0.01\,\mu g\,g^{-1}$ $(n = 6)$ giving a dopamine:NA

ratio of $0.5 \pm 0.09\%$. The difference between these two ratios is statistically significant at the 0.05 level.

Discussion

The abolition by TTX of both NA and dopamine release indicates both are dependent on nerve action potentials and almost certainly both are derived from nerves. In the vas deferens little or no dopamine appears to be extraneuronal. The question remains whether this dopamine exists as a precursor in noradrenergic nerves or in a small proportion of dopaminergic nerves. This point was most extensively investigated in the guinea-pig where two sets of results bear on this question. First, increasing the release of noradrenaline by up to 7 times by TEA or Pbz, drugs known to act on adrenergic nerves but through quite different mechanisms produced a similar increase in dopamine release so that the dopamine:NA remained constant. This suggests, though it does not prove, that the dopamine comes from the same adrenergic neurones as the NA. An alternative but, we believe, less likely explanation, is that there are dopaminergic nerves and these are influenced by TEA and Pbz in exactly the same way and to exactly the same extent as adrenergic nerves. For Pbz this seems unlikely. The increase in NA overflow by this drug is usually attributed to block of presynaptic α-receptors responsible for the negative feedback regulation and, less importantly, to blocking neuronal and extraneuronal uptake of noradrenaline. In a dopaminergic nerve there would be no noradrenaline release to activate the α-feedback system and the neuronal uptake of dopamine also differs

from that of NA. In these circumstances, it is unlikely Pbz could as successfully increase release from dopaminergic as from noradrenergic nerves. It has been suggested that the increase in overflow by Pbz may be due to some action of the drug other than block of presynaptic α-receptor (Bell, 1980; Holman & Suprenant, 1980). If this is so, then this mechanism also would have to be quantitatively equally important in noradrenergic and dopaminergic nerves.

The other observations bearing on the type of nerve releasing dopamine is the difference in the proportion of dopamine in the overflow from transmural stimulation and that in the tissue amine stores. While the proportion of dopamine in the overflow reflects the difference in the proportion of this amine in the stores in the two species, it is a distorted reflection so that for both species the dopamine levels were higher than expected. For example, the average dopamine percentage in the rat vas is 2% (Bell & Gillespie, 1981), whereas the overflow contained over 6%. Similarly the guinea-pig, whose tissue stores contain much less dopamine (in the present experiments about 0.8%), consistently produced values of about 1.3% in the nerve stimulated overflow. It is possible, but unlikely, that the overflow values are not a true reflection of the proportion of dopamine in the transmitter released from the nerves due to selective neuronal or extraneuronal uptake of NA as it diffused through the tissues to reach the bath fluid. However, since drugs that block NA uptake and metabolism made no difference to the ratio of dopamine to NA overflowing and since Pbz itself blocks both neuronal uptake, this possibility seems unlikely. On the assumption that the proportions of the two amines in the overflow do reflect their release, then the higher proportion of dopamine might suggest this amine does, indeed, come from nerves other than the adrenergic nerves. An alternative is that the dopamine and total NA in the tissue stores are not equally available to the nerve impulse. There is evidence that part of the vesicular NA is present in a rapidly equilibrating store available to the nerve impulse and part in a slowly equilibrating store (possibly the vesicle dense core) which is less available. Dopamine is present in the rapidly equilibrating store (Klein & Lagercrantz, 1982). If transmitter is liberated from such a labile pool, then the dopamine concentration might reflect its proportion in that pool assuming both amines are equally readily released. Expressed as a fraction of total tissue NA there would appear to be a preferential release of dopamine. For the same reason it would be the labile pool which would be depleted by repeated trains of stimulation and again the degree of depletion within that pool would be equal for the two amines but as a fraction of the total there would be preferential depletion of dopamine, which was our finding. Taken together with the results with TEA and Pbz we believe the likeliest explanation is that almost all of the dopamine within the vas deferens and all released by transmural nerve stimulation comes from the adrenergic nerve.

References

BELL, C. (1980). Effects of dopamine on adrenergic neuromuscular transmission in the guinea-pig vas deferens. *Br. J. Pharmac.*, **68**, 505–512.

BELL, C. (1982). Dopamine as a post-ganglionic autonomic neurotransmitter. *Neuroscience*, **7**, 1–8.

BELL, C. & GILLESPIE, J.S. (1981). Dopamine and noradrenaline levels in peripheral tissues of several mammalian species. *J. Neurochem.*, **36**, 703–706.

CALLINGHAM, B.A. & BARRAND, M.A. (1976). Catecholamines in blood. *J. Pharm. Pharmac.*, **28**, 356–360.

CHRISTENSEN, N.J., MATHIAS, C.J. & FRANKEL, H.L. (1976). Plasma and urinary dopamine: Studies during fasting and exercise and in tetraplegic man. *Eur. J. clin. Invest.*, **6**, 403–409.

HOLMAN, M.E. & SUPRENANT, A. (1980). An electrophysiological analysis of the effects of noradrenaline and α receptor antagonists on neuromuscular transmission in mammalian muscular arteries. *Br. J. Pharmac.*, **71**, 651–661.

KLEIN, R.L. & LAGERCRANTZ, H. (1981). Noradrenergic vesicles: Composition and function. In *Chemical Neurotransmission 75 years*, ed. Stjärne, L., Hedqvist, P., Lagercrantz, H. & Wennmalm, A. pp. 69–83. London: Academic Press.

KVETNANSKY, R., SUN, C.L., LAKE, C.R., THOA, N., TORDA, T. & KOPIN, I.J. (1978). Effect of handling and forced immobilization on rat plasma levels of epinephrine, norepinephrine and dopamine-β-hydroxylase. *Endocrinology*, **103**, 1868–1874.

LIBET, B. (1979). Dopaminergic synaptic processes in the superior cervical ganglion: Models for synaptic actions. In *The Neurobiology of Dopamine*, ed. Horn, A. S., Korf, J. & Westerink, B.H.C. pp. 453–474. London: Academic Press.

MUSACCHIO, J.M., FISCHER, J.E. & KOPIN, I.J. (1966). Subcellular distribution and release by sympathetic nerve stimulation of dopamine and α-methyl dopamine. *J. Pharmac. exp. Ther.*, **152**, 51–55.