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A comparison between the pattern of dopamine and noradrenaline release from sympathetic neurones of the dog mesenteric artery

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1 The release of dopamine and noradrenaline (NA), from the main trunk of the mesenteric artery and its proximal branches elicited by electrical nerve stimulation and K^+, has been measured by using high pressure liquid chromatography with electrochemical detection.

2 Both stimuli released dopamine and NA. With the main trunk of the mesenteric artery, dopamine represented 8% of the NA tissue content; the dopamine/NA ratio in the catecholamine overflow caused by nerve stimulation or K^+-induced depolarization also averaged 8%. For the proximal branches the tissue dopamine/NA ratio was significantly greater than that observed to occur in the overflow caused by nerve stimulation and K^+.

3 When the perifusion with a K^+-enriched medium was extended to 120 min the amount of NA released from both the main trunk and the proximal branches progressively declined. The same pattern of release was observed for dopamine in the main trunk, whereas for the proximal branches dopamine overflow did not decline throughout the perifusion period. The addition of α-methyl-*p*-tyrosine did not change the pattern of amine overflow.

4 Our interpretation of these results is that both dopamine and NA are derived from the same sympathetic neurone. In the proximal branches of the mesenteric artery dopamine and NA appear to be in two different storage structures, whereas in the main trunk both dopamine and NA are located in only one storage structure.

Introduction

In previous studies we have identified a noradrenaline-independent dopamine pool, as defined by selective 6-hydroxydopamine depletion of noradrenaline (NA), in the proximal branches of the mesenteric artery but not in the main trunk from the same blood vessel (Soares-da-Silva & Davidson, 1985; Caramona & Soares-da-Silva, 1985). Further investigations on this subject, in order to define the nature and cellular localization of this NA-independent dopamine pool, have provided evidence against the presence of independent dopaminergic neurones in this vascular area and indicated that this NA-independent dopamine pool is probably a non-precursor store of dopamine in noradrenergic neurones supplying the proximal branches of the mesenteric artery (Soares-da-Silva, 1986a, b). Taken together these results led us to suggest that in the proximal branches of the mesenteric artery the non-precursor dopamine pool and NA, though present in the very same neurone, are stored in two different storage structures; whereas in the main trunk of the mesenteric artery both dopamine and NA are probably stored in one common storage structure, since no evidence has been found in favour of a non-precursor dopamine pool (Soares-da-Silva, 1986b).

Although other evidence is needed in order to confirm this theory, the resolution of this problem clearly has important implications, since, until recently, dopamine in peripheral tissues was assumed to be a precursor for the synthesis of ß-hydroxylated catecholamines in adrenergic neurones and the adrenal medulla or a transmitter in its own right in independent dopaminergic neurones distributed to some well-defined areas (Bell, 1982). Thus, if there is a different storage structure for the non-precursor dopamine pool in noradrenergic neurones supplying the proximal branches of the mesenteric artery, but in the main trunk from the same blood vessel both dopamine and NA are located in one common storage structure, the proportional release of dopamine and NA in these two vascular tissues should vary according to the amine storage structure mobilized.

We have investigated this problem by studying the amount of both amines released from the main trunk of the mesenteric artery and its proximal branches during electrical stimulation or K^+-induced depolarization.

Methods

Mongrel dogs of either sex weighing 14–23 kg were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v., injected in the forelimb) and the main trunk of the anterior mesenteric artery and its proximal branches removed, stripped of their mesentery, rinsed free from blood and cut longitudinally. Each segment, weighing about 40 mg in the case of proximal branches of the mesenteric artery or up to 100 mg for samples of the main trunk and 4 cm long, was incubated for 30 min in 5 ml of Krebs solution, at 37°C, gassed with 95% O_2 and 5% CO_2, in the presence of 55 μM hydrocortisone and 0.1 mM pargyline (in order to block extraneuronal uptake and monoamine oxidase, respectively). 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 glucose 11. EDTA 0.04 mM was added to the Krebs solution in order to prevent oxidative destruction of catecholamines.

After the incubation period, segments of proximal branches and main trunk of the mesenteric artery were continuously perifused for 90 min in a 1 ml organ bath; aerated (95% O_2 and 5% CO_2) and warmed (37°C) Krebs solution (containing hydrocortisone as above) was pumped through the bath by means of a Harvard Peristaltic Pump (model 1210) at a constant rate of 0.3 ml min^{-1}, and the overflow was collected. In all experiments cocaine (10 μM) was added to the perifusion fluid from 0 min onwards. In some experiments phentolamine (0.2 μM) was also added at this time. Electrical stimulation (from t = 60 to t = 90 min) was applied by means of thin platinum electrodes attached to both ends of the preparation. The stimuli were rectangular pulses of alternating polarity, 2 ms duration, 100 V intensity at 1 Hz frequency (Hugo Sachs Stimulator, model 215/II). The fluid was collected in 10 ml cooled vials containing 0.8 ml 1.0 mM perchloric acid. At the end of the collection period 50 mg alumina was added and the pH of the sample immediately adjusted to pH 8.6. Mechanical shaking for 10 min was followed by centrifugation and the supernatant discarded. The adsorbed catecholamines were then eluted from the alumina with 150 μl 0.1 mM perchloric acid on Millipore microfilters (MF 1); 50 μl of the eluate was injected into a high pressure liquid chromatograph with electrochemical detection (BAS model 304 LC 4B) and the dopamine and NA measured. A 5 μM ODS column of 25 cm length was used. The mobile phase was degassed solution of monochloroacetic acid (0.15 mM), sodium octylsulphate (0.3 mM) and EDTA (2 mM), pH 3.0, pumped at a rate of 1.8 ml min^{-1}. A carbon paste electrode was used and the detector potential was + 0.65 V. Dihydroxybenzylamine was used as an internal standard. Peak height increased linearly with the concentration of NA and dopamine. The interassay coefficient of variation was less than 5%. Under our conditions, the lower limits of detection for NA and dopamine were 10 and 30 pg per sample, respectively.

The spontaneous loss from t = 30 to t = 60 min (in the absence of nerve stimulation) was also measured. This value was subtracted from the nerve-stimulation overflow, allowing the overflow as a result of nerve stimulation to be calculated.

After the stimulation period tissues were removed from the organ bath, blotted with filter paper, weighed, minced with fine scissors and homogenized with a Duall-Kontes homogenizer in 2.0 ml 0.1 mM perchloric acid. The homogenates were centrifuged (10000 r.p.m., 15 min, 0°C) and the supernatant decanted. Aliquots of 1.5 ml supernatant were placed in 5 ml conical glass vials with 50 mg alumina and the pH adjusted to 8.6. Mechanical shaking for 10 min was followed by centrifugation, the supernatant discarded and subsequently treated like samples of bathing fluid.

In another set of experiments tissues were perifused with a K^+-enriched Krebs solution; 40% of the NaCl was replaced by KCl in the KCl-enriched medium giving final concentrations of NaCl and KCl of 71 and 52 mM, respectively. Throughout these experiments, the main trunk of the mesenteric artery and its proximal branches were handled as described above, except that from t = 60 to t = 90 min or to t = 180 min K^+-enriched Krebs solution was pumped through the perifusion organ bath; from t = 0 to t = 60 min tissues were perifused with normal Krebs solution. Similar to the nerve stimulation experiments the spontaneous loss from t = 30 to t = 60 min was also measured and this value subtracted from the K^+-induced release to give the extra overflow as a result of catecholamine release during the depolarization period.

Differences between two means were estimated by Student's *t* test for unpaired data; a probability of less than 0.05 was assumed to denote a significant difference.

Drugs

Drugs used were: cocaine hydrochloride (Uquipa, Lisboa, Portugal), dopamine hydrochloride (Sigma, St. Louis, MO, U.S.A.), ethylenediaminetetracetic acid disodium salt (EDTA, Sigma), hydrocortisone

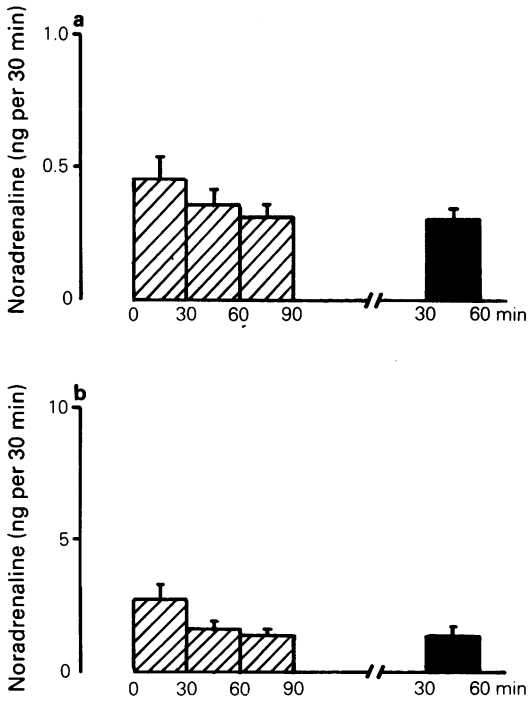

Figure 1 Progressive decline with time of the spontaneous loss of noradrenaline in (a) the main trunk of the mesenteric artery and (b) its proximal branches. The hatched columns represent the means of three experiments and the solid columns represent the mean noradrenaline spontaneous loss in ten similar experiments at least 30 min after setting up the preparations.

phosphate (Sigma), 1-α-methyl-*p*-tyrosine (Sigma), (−)-noradrenaline bitartrate (Sigma), pargyline hydrochloride (Sigma) and phentolamine hydrochloride (Regitin, Ciba, Switzerland).

Results

Spontaneous loss and nerve stimulated release

Both dopamine and NA were detectable in samples of the perifusion fluid obtained during nerve stimulation, but not in spontaneous overflow, where only NA was found. The amounts were greatest immediately after setting up the preparations and declined to a lower stable value from t = 30 to t = 60 min. In preliminary experiments the collection period was extended to t = 90 min. Although the spontaneous loss of NA appeared to be slightly decreased from t = 60 to t = 90 min, the difference was not found to be significant (Figure 1).

Electrical stimulation increased, by ten times, the NA overflow and dopamine was also released from both preparations under study, the main trunk of the mesenteric artery and its proximal branches. As shown in Table 1, the amount of NA released during nerve stimulation of the main trunk of the mesenteric artery was about three times less than that released from the proximal branches. However, when NA overflow values are expressed as fractional release no obvious difference was found between the two tissues studied (data not shown). The proportion of dopamine to NA released, which averaged 8.2% for the main trunk of the mesenteric artery, was about half that value for the proximal branches. Even when values of the released dopamine were expressed as a fraction of the tissue amine content a significant difference was found between the main trunk of the mesenteric artery and its proximal branches. Quite unexpectedly, whereas for the main trunk of the mesenteric artery both dopamine/NA ratios in tissue and nerve stimulation overflow averaged 8%, in the proximal branches the tissue dopamine/NA ratio was greater than that for nerve stimulation overflow ($P < 0.05$).

In another five experiments phentolamine was added to the perifusion fluid. Phentolamine had an increasing effect on the overflow of both amines during nerve stimulation of both tissues. However, in the main trunk phentolamine-induced increase of NA and dopamine release was of about 50 and 60%, respectively. In the proximal branches the effect of phentolamine was considerably more pronounced. The proportion of dopamine to NA in the overflow remained unchanged in the presence of phentolamine compared to control conditions (Table 1).

Spontaneous loss and K^+-induced release

The experiments where the main trunk of the mesenteric artery and its proximal branches were exposed to a K^+-enriched Krebs solution from t = 60 to t = 90 min showed that both dopamine and NA were released. As in experiments with nerve stimulation, dopamine was not found to occur in detectable amounts in spontaneous overflow (Table 2). Dopamine and NA overflow levels in experiments with K^+-enriched medium were similar to those for nerve stimulation; dopamine/NA ratios were about the same. With the main trunk of the mesenteric artery the proportion of dopamine to NA released by K^+ was the same as that found in the tissue. However, for the proximal branches the proportion of dopamine to NA released was lower than that found in the tissue (Table 2).

In five further experiments, the effect of phentolamine was examined. Phentolamine increased the

Table 1 Absolute and relative contents of dopamine (DA) and noradrenaline (NA) (ng g^{-1}) and spontaneous loss and nerve stimulated release of DA and NA (ng per 30 min) from (a) the main trunk of the mesenteric artery and (b) its proximal branches

	Spontaneous loss	*Nerve stimulation release*	*Tissue levels*
a *Main trunk of the mesenteric artery*			
NA	0.3 ± 0.04	4.1 ± 0.3	1214.8 ± 40.2
DA	-	0.3 ± 0.03	96.6 ± 6.1
DA/NA × 100	-	8.2 ± 1.3	8.0 ± 0.7
Phentolamine (0.2 μM) present throughout			
NA	0.4 ± 0.04	6.3 ± 0.7	1229.4 ± 115.8
DA	-	0.5 ± 0.06	83.4 ± 6.5
DA/NA × 100	-	8.0 ± 0.8	7.5 ± 0.7
b *Proximal branches of the mesenteric artery*			
NA	1.2 ± 0.11	13.4 ± 0.4	3403.7 ± 70.9
DA	-	0.4 ± 0.04	178.4 ± 17.0
DA/NA × 100	-	3.0 ± 0.35*	5.5 ± 0.4
Phentolamine (0.2 μM) present throughout			
NA	1.5 ± 0.18	28.6 ± 1.5**	3457.0 ± 105.3
DA	-	1.1 ± 0.18**	194.9 ± 18.3
DA/NA × 100	-	4.1 ± 0.3*	5.8 ± 0.5

Values are means ± s.e.mean of five experiments.
**Significantly different from control values ($P < 0.01$).
*Significantly different from values for tissue DA/NA ratios ($P < 0.05$).

overflow of both amines by about two times in the proximal branches, but the overflow dopamine/NA ratio remained almost the same as under control conditions. Interestingly, in the main trunk of the mesenteric artery, just as with nerve stimulation, phentolamine increased both dopamine and NA release by 50 and 60%, respectively. The overflow dopamine/NA ratio remained constant at 8%.

Table 2 Absolute and relative contents of dopamine (DA) and noradrenaline (NA) (ng g^{-1}) and spontaneous loss and K^+-evoked release of DA and NA (ng per 30 min) from (a) the main trunk of mesenteric artery and (b) its proximal branches

	Spontaneous loss	*K^+-evoked release*	*Tissue levels*
a *Main trunk of the mesenteric artery*			
NA	0.3 ± 0.03	5.2 ± 0.4	1272.0 ± 164.0
DA	-	0.4 ± 0.04	107.4 ± 11.9
DA/NA × 100	-	7.5 ± 0.7	8.2 ± 0.7
Phentolamine (0.2 μM) present throughout			
NA	0.3 ± 0.03	7.4 ± 0.6	1367.2 ± 105.3
DA	-	0.6 ± 0.05	105.4 ± 10.6
DA/NA × 100	-	8.0 ± 0.8	7.8 ± 0.6
b *Proximal branches of the mesenteric artery*			
NA	1.0 ± 0.09	13.8 ± 1.4	3315.4 ± 393.3
DA	-	0.5 ± 0.04	184.5 ± 18.4
DA/NA × 100	-	3.4 ± 0.30*	5.5 ± 0.5
Phentolamine (0.2 μM) present throughout			
NA	1.1 ± 0.10	26.4 ± 2.5**	3226.6 ± 282.9
DA	-	1.0 ± 0.09**	173.2 ± 18.8
DA/NA × 100	-	3.5 ± 0.3*	5.4 ± 0.5

Values are means ± s.e.mean of five experiments.
**Significantly different from control values ($P < 0.01$).
*Significantly different from values for tissue DA/NA ratio ($P < 0.05$).

Table 3 Absolute and relative contents of dopamine (DA) and noradrenaline (NA) ($ng\,g^{-1}$) and the effect of prolonged K^+-induced depolarization on the DA and NA overflow (ng per 30 min) from (a) the main trunk of the mesenteric artery and (b) its proximal branches

	Spontaneous loss	*DA and NA overflow in each period of depolarization*				*Tissue levels*
		First	*Second*	*Third*	*Fourth*	
a *Main trunk of the mesenteric artery*						
NA	0.4 ± 0.04	6.8 ± 0.7	4.6 ± 0.5*	3.8 ± 0.4**	3.0 ± 0.3**	1089.0 ± 90.3
DA	-	0.5 ± 0.06	0.3 ± 0.03*	0.2 ± 0.02**	0.2 ± 0.02**	94.3 ± 9.8
DA/NA × 100	-	8.1 ± 0.8	7.5 ± 0.8	6.1 ± 0.6	6.5 ± 0.6	8.5 ± 0.9
b *Proximal branches of the mesenteric artery*						
NA	0.9 ± 0.1	27.3 ± 1.9	12.6 ± 1.3**	10.2 ± 1.1**	8.3 ± 0.9**	2698.0 ± 70.9
DA	-	0.8 ± 0.07	0.9 ± 0.08	1.1 ± 0.1*	1.1 ± 0.09*	228.0 ± 29.7
DA/NA × 100	-	3.2 ± 0.3	7.3 ± 0.7	10.5 ± 1.2**	13.3 ± 0.4**	8.4 ± 0.9

Values are means ± s.e.mean of five experiments.
Significantly different from values for the first period of depolarization, $*P<0.05$; $**P<0.01$.

The effect of prolonged K^+-induced depolarization on dopamine and noradrenaline overflow

We extended the perifusion period with the K^+-enriched Krebs solution from t = 60 to t = 180 min and the overflow was collected in four equal fractions. In these experiments phentolamine (0.2 μM) was added to the perifusion fluid from t = 0 min onwards. As shown in Table 3, the amount of NA released, both from the main trunk of the mesenteric artery and its proximal branches, progressively declined in each period of depolarization so that in the fourth the overflow was only 40% of the first. With the main trunk the amount of dopamine released also progressively declined from the first period of depolarization to the fourth, as did NA. However, for the proximal branches, dopamine overlfow values progressively increased from the first to the last sample obtained during depolarization.

In the main trunk of the mesenteric artery the overflow dopamine/NA ratios were similar at all collection periods, though a slight decrease of the overflow dopamine/NA ratio was observed during the course of the experiment. On the other hand, for the proximal branches the overflow dopamine/NA ratios progressively increased from the first to the fourth period of depolarization.

The effect of α-methyl-p-tyrosine on the K^+-evoked release of dopamine and noradrenaline

The results in the preceding paragraph, namely those concerning dopamine overflow from the proximal branches of the mesenteric artery, led us to hypoth-

Table 4 Absolute and relative contents of dopamine (DA) and noradrenaline (NA) ($ng\,g^{-1}$) and the effect of α-methyl-*p*-tyrosine on the K^+-induced release of DA and NA (ng per 30 min) from (a) the main trunk of the mesenteric artery and (b) its proximal branches

	Spontaneous loss	*DA and NA overflow in each period of depolarization*				*Tissue levels*
		First	*Second*	*Third*	*Fourth*	
a *Main trunk of the mesenteric artery*						
NA	0.4 ± 0.04	6.1 ± 0.6	3.5 ± 0.3*	2.5 ± 0.3*	1.9 ± 0.2*	1033.9 ± 97.4
DA	-	0.4 ± 0.04	0.2 ± 0.02*	0.15 ± 0.02*	0.2 ± 0.02*	39.4 ± 3.8
DA/NA × 100	-	6.0 ± 0.6	5.8 ± 0.6	6.2 ± 0.6	5.3 ± 0.5	3.8 ± 0.4
b *Proximal branches of the mesenteric artery*						
NA	0.8 ± 0.09	15.9 ± 0.5	8.7 ± 0.4*	6.6 ± 0.3*	5.2 ± 0.3*	2344.2 ± 210.4
DA	-	0.4 ± 0.03	0.4 ± 0.04	0.4 ± 0.06	0.4 ± 0.04	44.8 ± 6.7
DA/NA × 100	-	2.5 ± 0.2	4.5 ± 0.4*	4.5 ± 0.3*	7.9 ± 1.0*	1.9 ± 0.2

Values are means ± s.e.mean of five experiments.
Significantly different from values for the first depolarization period, $*P<0.01$.

esize that an increase in catecholamine synthesis was occurring during the prolonged depolarization to which tissues were submitted. For this reason experiments with α-methyl-*p*-tyrosine (which inhibits dopamine and NA synthesis) were performed. α-Methyl-*p*-tyrosine (2 μM) was added to the perifusion fluid from t = 0 min onwards, and the experimental arrangement was similar to that described in the preceding paragraph.

For the proximal branches of the mesenteric artery, α-methyl-*p*-tyrosine produced a significant decrease of both dopamine and NA overflow values at all four periods of depolarization. However, for the main trunk both dopamine and NA overflow levels only became significantly reduced after t = 90 min, as shown in Table 4. Contrary to the progressive decline of NA overflow from the first to the fourth period of depolarization, in the proximal branches of the mesenteric artery dopamine overflow remained unchanged throughout the perifusion period despite the inhibition of catecholamine synthesis.

Surprisingly, only the dopamine content in both blood vessels was found to be significantly decreased after α-methyl-*p*-tyrosine, whereas NA content was unaffected.

Discussion

Nerve stimulation and K^+-induced depolarization caused a simultaneous release of both dopamine and NA. However, for the proximal branches of the mesenteric artery the proportion of dopamine released was signficantly lower than that found in the tissue, whereas for the main trunk the proportion of dopamine to NA released was similar to that found in the tissue. Therefore, it is possible that in the proximal branches of the mesenteric artery dopamine and NA do not constitute a homogeneous mixture.

The most important result needed to elucidate whether dopamine is present in neurones or in extraneuronal structures is that phentolamine, a drug known to act on adrenergic nerves, produced a similar increase of dopamine and NA release, so that the proportion of dopamine to NA in the overflow remained unchanged. This also suggests, though it does not prove, that dopamine and NA are released from the same neurone. As is well known, evidence favours the view that the increase in NA overflow by phentolamine is due to blockade of α-adrenoceptors present on sympathetic nerve terminals (Langer, 1977; Starke, 1977). In a dopaminergic nerve terminal there would be no NA release to activate the α-adrenoceptor mediated feedback system. Therefore, it is unlikely that dopamine comes from dopaminergic nerves but almost certain that both dopamine and NA are released from the same sympathetic neurone. This also agrees with previous results, using a different experimental approach, where evidence against the presence of independent dopaminergic neurones in this vascular area was presented (Soares-da-Silva, 1986a, b).

Assuming that the proportion of the two amines in the overflow from the proximal branches does reflect their release, then the higher proportion of NA might suggest that dopamine and NA in tissue stores are equally mobilized by nerve stimulation and K^+-induced depolarization. Expressed as a fraction of total tissue amine there is in fact a preferential release of NA. In addition, it has been shown that part of the total NA in tissue stores is present in a pool which is available to be depleted rapidly by nerve impulses and another pool which is less available for depletion (Trifaró & Cubeddu, 1979; Fried, 1980). Thus, if NA is mainly released from the former pool there would be a preferential release of NA and for the same reason it would be this pool which would be depleted by continuous K^+-induced depolarization, which was our finding. In contrast to the results with NA, dopamine overflow in the same blood vessel progressively increased or remained about the same when tyrosine hydroxylase was inhibited throughout the perifusion period, thus suggesting that a pool less available for depletion is probably the dominating storage form for dopamine. Thus, the possibility that there are two different types of storage structures for dopamine and NA inside sympathetic nerves supplying the proximal branches of the mesenteric has to be faced.

For the main trunk of the mesenteric artery, where all of the dopamine is a precursor for NA (Soares-da-Silva, 1986a, b), similar dopamine/NA ratios in the overflow and tissue stores might suggest that dopamine and NA in the tissue are equally available for the nerve impulse. These results agree with those found by Bell *et al.* (1984) for the rat and guinea-pig vas deferens, where innervation is purely noradrenergic (Bell & Gillespie, 1981) and both dopamine and NA are released from a common store during noradrenergic transmission.

The experiments with α-methyl-*p*-tyrosine also agree with the view that in the proximal branches of the mesenteric artery dopamine and NA in tissue stores are not equally mobilized by depolarization and dopamine is probably stored in a pool which is only slowly depleted, though more sensitive to tyrosine hydroxylase inhibition than the NA pool. These experiments further suggest that catecholamine synthesis occurs during K^+-induced depolarization of the dog mesenteric artery and also provide evidence in favour of a preferential release of newly synthesized amine, as desribed by Kopin *et al.* (1968) and Klein & Harden (1975). On the other hand, data from experiments with α-methyl-*p*-tyrosine also indicate that

the dopamine pool (slowly depleted), just as the NA pool (rapidly depleted), reside in cellular compartments equally involved in transmitter turnover. In addition, they agree with the occurrence of active catecholamine synthesis during *in vitro* nerve activation, as extensively reviewed by Weiner (1970).

Although it is not clear what role the dopaminergic mechanisms play in cardiovascular homeostatis and pathophysiology of cardiovascular disorders, there is evidence to suggest an involvement of the catecholamine dopamine in conditions such as hypertension, heart failure and cardiac ischaemia. In fact, the activity of the renin-angiotensin-aldosterone system may be regulated by the endogenous dopaminergic tone, since dopamine receptor antagonists modulate the aldosterone response to angiotensin II (A II) (Gordon *et al.*, 1983). On the other hand, at the advanced hypertensive stage, the effects of dopamine ß-hydroxylase and phenylethanolamine N-methyltranferase in spontaneously hypertensive rats (SHR) remained low, whereas that of tyrosine hydroxylase increased, indicating a non-uniform change in the synthetic enzyme activities during the course of hypertension (Grobecker *et al.*, 1982). A similar situation has been described in the failing hamster heart; increased tyrosine hydroxylase and decreased dopamine ß-hydroxylase activity with increased dopamine tissue content (Sole *et al.*, 1982). Other authors have observed increased tissue dopamine levels in the heart, kidney, adrenal medualla (Racz *et al.*, 1986) and sympathetic ganglia (Gianutsos & Moore, 1978). Also, increased release of dopamine from sympathetic nerves was observed in perinephritic hypertensive dogs (Branco & Osswald, 1986) and ischaemic myocardium of the rat (Schömig *et al.*, 1984). Moreover, it has been suggested that the down regulation of dopamine receptors in SHR (Beck & Sowers, 1984) could be due to an increased release of dopamine from sympathetic nerves (Racz *et al.*, 1986).

In conclusion, our interpretation of the results presented is that both dopamine and NA are derived from the same sympathetic neurones. In the proximal branches of the mesenteric artery dopamine and NA appear to be in two different storage structures with a different pattern of release which suggest that dopamine might play a role in mesenteric vascular homeostasis. In fact, preliminary reults have suggested that dopamine in this vascular area may have a functional role in controlling transmitter release since dopamine receptor antagonists increased NA outflow (Soares-da-Silva, 1986c). Thus it would be interesting to perform further investigations under conditions of altered cardiovascular homeostasis such as those occurring in hypertension and heart failure.

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