Br. J. Pharmacol. (1988), **95**, 218–224

Evidence for dopaminergic co-transmission in dog mesenteric arterial vessels

P. Soares-da-Silva

Laboratório de Farmacologia, Faculdade de Medicina, 4200 Porto, Portugal

1 The overflow of dopamine and noradrenaline (NA) from the main trunk of the dog mesenteric artery and its proximal branches during prolonged depolarization (120 min) by K^+ (52 mM) was quantified by high performance liquid chromatography with electrochemical detection.

2 K^+-induced depolarization resulted in release of both dopamine and NA. The amount of NA released from both blood vessels declined progressively throughout the experiment. In the main trunk the same pattern of release was observed for dopamine, whereas in the proximal branches the overflow of dopamine increased throughout the experiment.

3 The addition of phentolamine (0.2 μM) to the perifusion fluid increased the overflow of both amines. In the presence of sulpiride (1 μM) the overflow of dopamine and NA was found to be increased in the proximal branches, but not in the main trunk. The addition of phentolamine to sulpiride caused a further increase in amine overflow in proximal branches, but not in the main trunk.

4 The addition of α-methyl-*p*-tyrosine (50 μM) to the perifusion fluid caused a decrease in the amounts of dopamine and NA released from both preparations. In α-methyl-*p*-tyrosine-treated preparations phentolamine increased amine overflow to the same extent as in experiments without tyrosine hydroxylase inhibition. The increasing effect of sulpiride on the overflow of dopamine and NA from the proximal branches was completely abolished after α-methyl-*p*-tyrosine.

5 The results presented suggest that in the proximal branches of the dog mesenteric artery, dopamine β-hydroxylase represents a rate limiting step in the synthesis of NA; dopamine, through activation of prejunctional dopamine receptors acts like a prejunctional co-transmitter in the control of transmitter release, but only newly-synthesized dopamine appears to be responsible for this effect.

Introduction

Nerve endings in the peripheral sympathetic nervous system are involved in the processes of synthesis, storage, release and uptake of the transmitter. The released transmitter not only activates postjunctional receptors to elicit the typical responses of effector tissues, but also activates receptors on the outer surface of the axoplasmic membrane. The transmitter thus released, on reaching a threshold concentration in the biophase, activates prejunctional receptors triggering a feedback mechanism that controls its further release. This has been found for noradrenaline (NA) in a variety of peripheral sympathetically innervated tissues and the activation of inhibitory prejunctional α-adrenoceptors by the released NA is thought to constitute a physiological mechanism that conserves the transmitter (Langer, 1981; Starke, 1987).

Dopamine is the immediate precursor of NA in sympathetic neurones and there is increasing evidence that the amine is co-released with NA during nerve activation (Bell *et al.*, 1984; Bradley & Hjemdahl, 1984; Soares-da-Silva, 1987a,b; 1988). Although it has been shown that the activation of prejunctional dopamine receptors can also lead to a decrease in transmitter release, most observations do not support the view that, under most conditions, released dopamine activates these receptors (Langer, 1981; Willems *et al.*, 1985). However, it has been suggested (Lokhandwala & Barrett, 1982) that under conditions of continuous nerve stimulation, where the amount of dopamine β-hydroxylase becomes rate limiting, greater amounts of newly synthesized dopamine would be released into the biophase. This would make possible the activation of prejunctional dopamine receptors by the released dopamine, acting as a prejunctional co-transmitter, further intensifying the negative feedback control of transmitter release activated by NA.

The question in which we were interested was to what extent does the co-released dopamine activate

the negative feedback control of transmitter release during prolonged nerve depolarization, reinforcing the effects of NA upon prejunctional α-adrenoceptors? For this purpose we have used the main trunk of the dog mesenteric artery and its proximal branches which appeared to be useful experimental tools since both segments of the mesenteric artery are densely innervated (Azevedo & Soares-da-Silva, 1981; Soares-da-Silva & Azevedo, 1985); endogenous dopamine and NA can be released from both arterial segments by either electrical stimulation or depolarization with K^+ (Soares-da-Silva, 1987a); dopamine β-hydroxylase is rate limiting in the synthesis of NA in the proximal branches but not in the main trunk (Soares-da-Silva, 1986) and inhibitory α-adrenoceptors and dopamine receptors are present in nerve endings of both segments of the mesenteric artery (Soares-da-Silva, 1987b).

The present paper describes our findings on the overflow of endogenous NA and dopamine from the main trunk of the mesenteric artery and its proximal branches during prolonged nerve depolarization by K^+ (52 mM) and the effect of blockade of prejunctional dopamine receptors and α-adrenoceptors on amine release. In some experiments α-methyl-*p*-tyrosine was added to the perifusion fluid, in order to prevent excessive accumulation of dopamine. If dopamine β-hydroxylase becomes rate limiting during prolonged depolarization by K^+, with accumulation of greater amounts of newly-synthesized dopamine, the inhibition of tyrosine hydroxylase would result in a decrease of dopamine in sympathetic nerve endings.

Methods

Mongrel dogs of either sex weighing 14–21 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 weighed 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 was 4 cm long. The segments were incubated for 30 min in 5 ml of Krebs solution (37°C), gassed with 95% O_2 and 5% CO_2, in the presence of 55 μM hydrocortisone and 100 μM 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, $NaCHO_3$ 25, KH_2PO_4 1.2 and glucose 11. EDTA 40 μM was added to the Krebs solution in order to prevent oxidation of catecholamines.

After the incubation period, segments of proximal branches and main trunk of the mesenteric artery were continuously perifused for 180 min in a 1 ml organ bath; gassed (95% O_2 and 5% CO_2) and warm (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}, overflow being collected. In all experiments cocaine (10 μM) and propranolol (1 μM) were added to the perifusion fluid from 0 min onwards. Tissues were perifused with a K^+-enriched Krebs solution from $t = 60$ to $t = 180$ min; 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. In another set of experiments the spontaneous overflow of both catecholamines from $t = 30$ to $t = 180$ min was also measured and these values subtracted from the overflow observed in the presence of K^+-enriched Krebs solution to obtain the depolarization-evoked overflow of NA and dopamine.

In another series of experiments tyrosine hydroxylase was inhibited. Tissues were collected in Krebs solution containng α-methyl-*p*-tyrosine (50 μM) and all subsequent steps in the preparation of tissues to be perifused was performed in a α-methyl-*p*-tyrosine containing medium; α-methyl-*p*-tyrosine was also present in the perifuson fluid.

In some experiments, either when α-methyl-*p*-tyrosine was present or not, phentolamine (0.2 μM), sulpiride (1 μM) or phentolamine plus sulpiride were added to the perifusion fluid from 0 min onwards.

The fluid was collected in 10 ml cooled vials containing 0.8 ml 1.0 M 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 M perchloric acid on Millipore microfilters (MF 1); 50 μl of the eluate was injected into a high performance liquid chromatograph with electrochemical detection (BAS model 304 LC 4A) and dopamine and noradrenaline measured. A 5 μM ODS column of 25 cm length was used. The mobile phase was degassed solution of citric acid (0.1 mM), sodium acetate (0.1 M), sodium octylsulphate (0.5 mM), EDTA (0.15 M), dibutylamine (1 mM) and methanol (10% v/v), pumped at a rate of 1.0 ml min^{-1}. A carbon paste electrode was used and the detector potential was +0.75 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 for detection of noradrenaline and

Table 1 Spontaneous overflow of noradrenaline (ng $30\,min^{-1}$) from control and α-methyl-*p*-tyrosine (AMPT, 50 μM) treated preparations from the main trunk of the dog mesenteric artery and its proximal branches in four overflow periods (30 min each)

		Overflow periods			
	Treatment	1st	2nd	3rd	4th
Main trunk					
	Control	0.3 ± 0.03	0.2 ± 0.03	0.2 ± 0.02	0.2 ± 0.02
	AMPT	0.1 ± 0.02*	0.1 ± 0.01*	ND	ND
Proximal branches					
	Control	1.1 ± 0.10	0.8 ± 0.09	0.5 ± 0.06	0.4 ± 0.04
	AMPT	0.5 ± 0.07*	0.3 ± 0.04*	0.1 ± 0.02*	ND

Values are means ± s.e.mean ($n = 5$).
* Significantly different from corresponding values of control ($P < 0.05$).
ND Not detectable.

dopamine were 10 and 30 pg per sample, respectively.

After the depolarization period, tissues were removed from the organ bath, blotted with filter paper, weighed, minced with fine scissors in 2.0 ml 0.1 M perchloric acid and the catecholamines measured as previously reported (Soares-da-Silva, 1987a).

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 hdyrochloride (Sigma, St. Louis, MO, U.S.A.), ethylenediaminetetracetic acid disodium salt (EDTA, Sigma), hydrocortisone phosphate (Sigma), 1-α-methyl-*p*-tyrosine (Sigma), (−)-noradrenaline bitartrate (Sigma), pargyline hydrochloride (Sigma), phentolamine hydrochloride (Regitin, Ciba, Switzerland), propranolol hydrochloride (Sigma) and RS-sulpiride (Sigma).

Results

Only NA was found in detectable amounts in samples of perifusion fluid during spontaneous overflow. As previously shown (Soares-da-Silva, 1987a), the amounts of NA in the spontaneous overflow were greatest immediately after setting up the preparations and declined to a lower stable value from $t = 30$ min onwards. Table 1 shows the spontaneous overflow of NA in the main trunk of the mesenteric artery and its proximal branches from $t = 60$ to $t = 180$ min. The addition of α-methyl-*p*-tyrosine to the perifusion fluid from $t = 0$ min onwards produced a significant reduction in the spontaneous overflow of NA (Table 1); no other drug used in the course of these experiments altered the spontaneous overflow of NA.

When the main trunk and its proximal branches were perifused with a K^+-enriched Krebs solution from $t = 60$ to $t = 180$ min both dopamine and NA were released. As shown in Figure 1, the amount of NA released, from the main trunk and the proximal branches, declined progressively from the first to the fourth depolarization period. For the main trunk, the pattern of dopamine depolarization-evoked overflow closely followed that of NA, whereas in the proximal branches the amounts of dopamine rel-

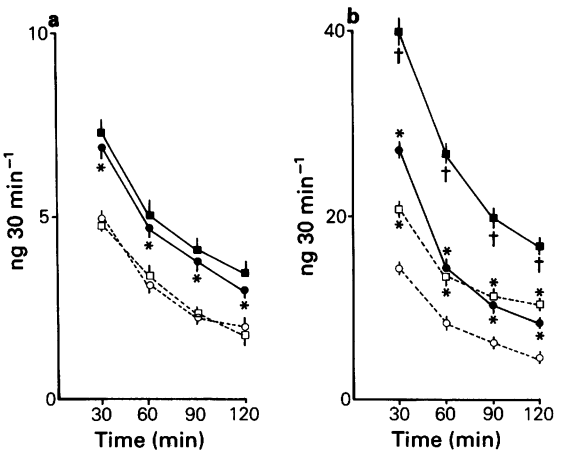

Figure 1 Absolute noradrenaline overflow levels from (a) the main trunk of the dog mesenteric artery and (b) its proximal branches during prolonged (120 min) K^+ (52 mM)-induced depolarization from controls (○) and the effect of 0.2 μM phentolamine (●), 1 μM sulpiride (□) or phentolamine plus sulpiride (■). Shown are means of five experiments per group with s.e.mean indicated by vertical lines. * Significantly different from control values ($P < 0.01$); † significantly different from values for phentolamine alone ($P < 0.01$).

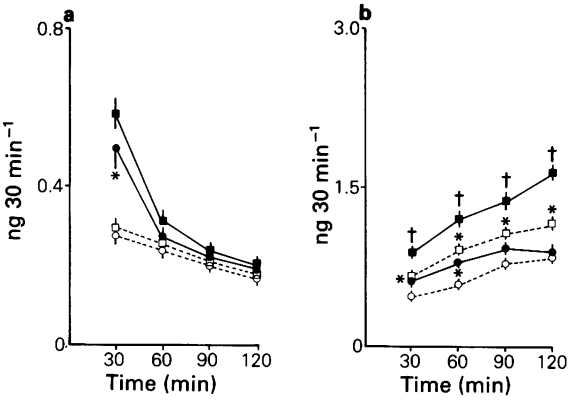

Figure 2 Absolute dopamine overflow levels from (a) the main trunk of the dog mesenteric artery and (b) its proximal branches during prolonged (120 min) K^+ (52 mM)-induced depolarization from controls (○) and the effect of 0.2 μM phentolamine (●), 1 μM sulpiride (□) or phentolamine plus sulpiride (■). Shown are means of five experiments per group with s.e.mean indicated by vertical lines. * Significantly different from control values ($P < 0.01$); † significantly different from values for phentolamine alone ($P < 0.01$).

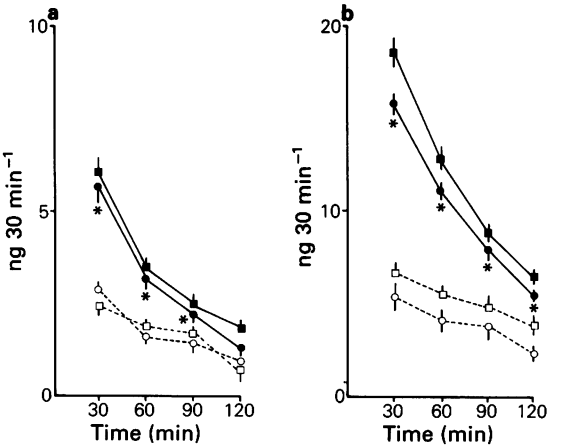

Figure 3 Absolute noradrenaline overflow levels from (a) the main trunk of the dog mesenteric artery and (b) its proximal branches during prolonged (120 min) K^+ (52 mM)-induced depolarization in α-methyl-*p*-tyroxine (50 μM) treated preparations from controls (○) and the effect of 0.2 μM phentolamine (●), 1 μM sulpiride (□) or phentolamine plus sulpiride (■). Shown are means of five experiments per group with s.e.mean indicated by vertical lines. * Significantly different from control values ($P < 0.01$).

eased increased progressively from the first to the last depolarization period (Figure 2).

The addition of phentolamine to the perifusion fluid produced a marked increase in the amount of NA and dopamine released from both arterial segments. For the main trunk the increasing effect of phentolamine on dopamine overflow was only observed in the first depolarization period (Figure 2). In the proximal branches phentolamine caused a significant increase in the overflow of NA, which was more pronounced in the first depolarization period, but still maintained in the last three periods (Figure 1); for dopamine the effect of α-adrenoceptors blockade was only observed in the two first periods of depolarization (Figure 2).

The blockade of prejunctional dopamine receptors by sulpiride did not alter dopamine and NA overflow or the pattern of amine overflow from the main trunk (Figures 1 and 2). Also, the addition of sulpiride to the phentolamine-containing perifusion medium did not produce a further increase in amine overflow. In contrast, in the proximal branches sulpiride enhanced the overflow of dopamine and NA in all four periods of depolarization; this effect was, however, more marked in the last two depolarization periods. When sulpiride and phentolamine were present in the perifusion fluid the increasing effect on amine overflow was greater than that observed with either drug alone (Figures 1 and 2).

The addition of α-methyl-*p*-tyrosine to the perifusion fluid caused a marked reduction in the amounts of dopamine and NA released. In the main trunk, when α-methyl-*p*-tyrosine was present, phentolamine produced a significant increase in the K^+-evoked overflow of NA in all depolarization periods, though it was more pronounced during the two first depolarization periods. Sulpiride alone or with added phentolamine did not cause any further increase in the overflow of NA, when compared with controls or in the presence of phentolamine alone respectively (Figure 3). As in the experiments without α-methyl-*p*-tyrosine, when tyrosine hydroxylase was inhibited, phentolamine did not alter the overflow of dopamine from the main trunk, except in the first and second depolarization periods where a significant increase in amine overflow was found to occur (Figure 4).

In the proximal branches, α-methyl-*p*-tyrosine produced a significant decrease in the release of both amines in a similar proportion to that found in the main trunk (50% reduction). Phentolamine significantly increased the release of NA in all four periods of depolarization; again, this effect was three times greater in the first depolarization period than in the last period (Figure 3). The increasing effect of phentolamine on the release of dopamine was found to occur in the two first depolarization periods only (Figure 4). In sulpiride-treated preparations NA and dopamine overflow was similar to that in controls. Also, the addition of sulpiride to the phentolamine

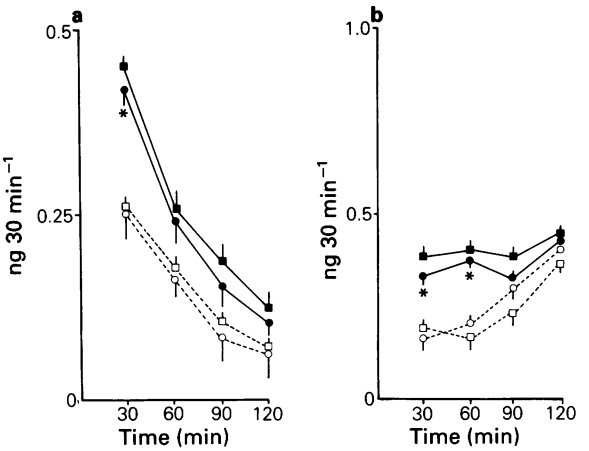

Figure 4 Absolute dopamine overflow levels from (a) the main trunk of the dog mesenteric artery and (b) its proximal branches during prolonged (120 min) K^+ (52 mM)-induced depolarization in α-methyl-*p*-tyrosine (50 μM) treated preparations from controls (○) and the effect of 0.2 μM phentolamine (●), 1 μM sulpiride (□) or phentolamine plus sulpiride (■). Shown are means of five experiments per group with s.e.mean indicated by vertical lines. * Significantly different from control values ($P < 0.01$).

containing Krebs solution did not cause a further increase in the release of dopamine and NA (Figures 3 and 4).

Tissue dopamine and NA contents in both blood vessels were similar in the different experimental groups. In the experiments where tissues were perifused with α-methyl-*p*-tyrosine a marked reduction in dopamine tissue content was found to occur (Table 2).

Discussion

The results presented show that catecholamine synthesis occurs during nerve depolarization and that in the proximal branches of the dog mesenteric artery, in contrast to the main trunk, dopamine β-hydroxylase is the rate limiting factor in the formation of newly-synthesized NA and, therefore, favours the accumulation of dopamine available for release. After being released into the biophase, the newly-synthesized dopamine acts as a prejunctional co-transmitter, activating prejunctional dopamine receptors which leads to a decrease in the K^+-evoked overflow of the amines.

As with α-adrenoceptors on nerve endings, activation of prejunctional dopamine receptors leads to a decrease in the overflow of NA and dopamine from sympathetic nerves, but the blockade of dopamine receptors does not usually produce an increase in the release of the amines (Langer, 1981; Lokhandwala & Barrett, 1982; Willems *et al.*, 1985). This has been observed in the majority of sympathetically-innervated tissues and one of the reasons is that the concentration of dopamine attained in the biophase is too low to stimulate the prejunctional dopamine receptors. As described previously (Soares-da-Silva, 1987b), only in the proximal branches was sulpiride

Table 2 Noradrenaline and dopamine content (in $ng\,g^{-1}$) of perifused tissues in the absence and presence of α-methyl-*p*-tyrosine (AMPT 50 μM)

Treatment	*Noradrenaline*	*Dopamine*
Main trunk		
Control	1551.8 ± 105.0	105.7 ± 8.4
Phentolamine	1389.0 ± 90.3	94.3 ± 9.8
Sulpiride	1689.3 ± 98.0	93.2 ± 8.2
Phentolamine + sulpiride	1633.6 ± 67.0	93.4 ± 8.4
AMPT	1344.7 ± 154.0	16.4 ± 2.2*
AMPT + phentolamine	1033.9 ± 97.4	39.4 ± 3.8*
AMPT + sulpiride	1275.4 ± 85.4	23.6 ± 2.8*
AMPT + phentolamine + sulpiride	1703.2 ± 156.0	20.4 ± 3.8*
Proximal branches		
Control	2569.0 ± 108.0	161.6 ± 22.7
Phentolamine	2698.0 ± 71.0	228.0 ± 29.7
Sulpiride	2508.4 ± 212.0	162.5 ± 17.3
Phentolamine + sulpiride	3226.3 ± 448.0	173.2 ± 28.8
AMPT	2989.9 ± 175.0	41.3 ± 7.2*
AMPT + phentolamine	2377.3 ± 210.0	44.8 ± 6.7*
AMPT + sulpiride	2431.0 ± 286.0	50.3 ± 4.8*
AMPT + phentolamine + sulpiride	2241.0 ± 160.0	35.9 ± 4.9*

Values are means ± s.e.mean ($n = 5$). * Significantly different from corresponding values of control ($P < 0.01$).

found to increase the K^+-evoked overflow of dopamine and NA. This unusual finding in sympathetic neurotransmission has been suggested to occur as a consequence of differences of amine compartmentation inside the nerves and its availability for release (Soares-da-Silva, 1987b). The amine thus stored would be in a unique position to act as a co-transmitter and further intensify the effects of NA upon the negative feedback mechanisms of control of transmitter release. Some of the criteria found to characterize dopamine as a co-transmitter in this vascular bed were that the amine is stored in a compartment different from that which contains NA (Soares-da-Silva & Davidson, 1985), a substantial proportion of dopamine is not converted to NA (Soares-da-Silva, 1986) and presents a pattern of release different from that of NA in conditions of electrical nerve stimulation and depolarization by K^+ (Soares-da-Silva, 1987a; 1988).

To some extent the results obtained in this study support previous findings and also clarify some of the unsolved questions. First, the increasing effect of sulpiride on the overflow of dopamine and NA was maintained throughout the depolarization period; its effect was even greater during the latter depolarization periods in which the largest amounts of dopamine were released. Second, the inhibition of tyrosine hydroxylase by α-methyl-*p*-tyrosine completely abolished the increasing effect of sulpiride on the overflow of dopamine and NA. These two findings suggest that sustained amounts of dopamine are being released from the proximal branches achieving concentrations high enough to activate the negative feedback system operated by prejunctional dopamine receptors. It appears, however, that only when catecholamine synthesis is taking place does dopaminergic co-transmission occur. The results presented suggest that dopamine β-hydroxylase is a rate limiting enzyme so that when nerves are depolarized during prolonged periods the overflow of NA progressively declines whereas that of dopamine progressively increases. Tyrosine hydroxylase inhibition drastically reduced the amounts of dopamine released. However, the rate limiting characteristic of dopamine β-hydroxylase in the synthesis of NA does not seem to explain why dopamine, even when tyrosine hydroxylase is inhibited, has a pattern of release different from that of NA. One possible explanation for this finding could be that of the storage of dopamine and NA in different neuronal compartments. In fact, we have recently reported that in the proximal branches, by contrast to the main trunk, a substantial proportion of large dense core vesicles (LDCV) and dopamine were not affected by 6-hydroxydopamine and that the depletion of NA was accompanied by an almost complete disappearance of small dense core vesicles (SDCV) (Soares-da-Silva & Azevedo, 1988). This would agree with the view that SDCV store a high proportion of NA and are the first vesicles mobilized during nerve activation (Basbaum & Heuser, 1979), whereas LDCV store high amounts of dopamine, as found to occur in other tissues (Neuman *et al.*, 1984; Muller & Bell, 1986), and are more difficult to mobilize during nerve activation (Basbaum & Heuser, 1979).

It is well known that inhibitors of dopamine β-hydroxylase produce a decrease in blood pressure in normotensive subjects and in hypertensive patients (Matta & Wooten, 1973). More recently, from results of experiments with a new dopamine β-hydroxylase inhibitor (SK&F 102698), it has been suggested that the antihypertensive effects of these compounds could partially depend on the activation of dopamine receptors in vascular smooth muscle cells by the released dopamine (Ohlstein *et al.*, 1987). In fact, if the cardiovascular effects of dopamine β-hydroxylase inhibitors are dependent upon an increased availability of dopamine inside the nerves, it might be expected that such compounds would also intensify the activation of prejunctional dopamine receptors and therefore decrease sympathetic tone.

The K^+-evoked overflow of dopamine from the main trunk closely follows the pattern of NA overflow and the amine concentrations achieved in the biophase do not appear to be high enough to activate the prejunctional dopamine receptors found in this vascular area (Soares-da-Silva, 1987b). The results obtained in this study also agree with the previous suggestion that in this blood vessel most of the dopamine is converted to NA (Soares-da-Silva, 1986). As a matter of fact, the decrease in the release of NA during depolarization was not as steep in this blood vessel as that observed in the proximal branches; this would suggest that some of the dopamine is hydroxylated to NA during depolarization. Also, in spite of active synthesis, the proportion of dopamine to NA released was similar in all periods of depolarization. This agrees with the suggestion that dopamine β-hydroxylase is not rate limiting in this artery, as also found by Bell *et al.* (1984) in the rat and guinea-pig vas deferens, even when tissues were submitted to prolonged nerve stimulation.

The magnitude of the increase in overflow of NA induced by phentolamine is somewhat related to the concentrations of the amine attained in the biophase, and constitutes one of the criteria to assess the functional role of the released transmitter in the control of sympathetic neurotransmission (Langer, 1981; Starke, 1987). It is then to be expected that when the concentrations of the released NA become lower, the effect of blockade of prejunctional α-adrenoceptors would be proportionally decreased. In agreement with this view are the results presented in this study,

showing that the effect of phentolamine in the release of NA from the main trunk and the proximal branches was greater in overflow periods during which large amounts of NA were released. When α-methyl-*p*-tyrosine was added to the perifusion fluid, the amounts of released NA were reduced by half in both blood vessels, but the effect of phentolamine on the release of the amine was of about the same magnitude as in experiments without tyrosine hydroxylase inhibition. Although these results apparently conflict with the classical view that proposes that the effect of prejunctional α-adrenoceptors blockade is related to the amounts of NA released into the biophase (Enero & Langer, 1973), one possible explanation is that during inhibition of tyrosine hydroxylase, supersensitivity of prejunctional α-adrenoceptors has developed. However, as mentioned for experiments without α-methyl-*p*-tyrosine, the effect of α-adrenoceptor blockade was two to three fold higher in the first than in the fourth depolarization period.

In conclusion, the results presented here suggest that dopamine β-hydroxylase is rate limiting in the synthesis of NA in the proximal branches, favouring the accumulation of larger amounts of dopamine to be released, which ultimately are responsible for the phenomenon of dopaminergic co-transmission. It would be interesting to see whether dopaminergic co-transmission operates in *in vivo* experimental conditions and whether this constitutes an additional mechanism to that of activation of prejunctional α-adrenoceptors in order to conserve the transmitter.

The skilful technical assistance of Miss M. Prazeres Cleto and Miss Manuela Moura is gratefully acknowledged. This work was supported by Instituto Nacional de Investigação Científica (Portugal).

References

AZEVEDO, I. & SOARES-DA-SILVA, P. (1981). Are fibroblasts adrenergically innervated cells? *Blood Vessels* **18**, 330–332.

BASBAUM, C.B. & HEUSER, J.E. (1979). Morphological studies of stimulated adrenergic axon varicosities in the mouse vas deferens. *J. Cell. Biol.*, **80**, 310–325.

BELL, C., GILLESPIE, J.S. & MACRAE, I. (1984). Release of noradrenaline and dopamine by nerve stimulation in the guinea pig and rat vas deferens. *Br. J. Pharmacol.*, **81**, 563–569.

BRADLEY, T. & HJEMDAHL, P. (1984). Further studies on renal nerve stimulation induced release of noradrenaline and dopamine from the canine kidney in situ. *Acta Physiol. Scand.*, **122**, 369–379.

ENERO, M.A. & LANGER, S.Z. (1973). Influence of reserpine-induced depletion of noradrenaline on the negative feed-back mechanism for transmitter release during nerve stimulation. *Br. J. Pharmacol.*, **49**, 214–225.

LANGER, S.Z. (1981). Presynaptic regulation of the release of catecholamines. *Pharmacol. Rev.*, **32**, 337–362.

LOKHANDWALA, M.F. & BARRETT, R.J. (1982). Cardiovascular dopamine receptors: Physiological, pharmacological and therapeutic implications. *J. Auton. Pharmacol.*, **3**, 189–215.

MATTA, R.J. & WOOTEN, G.F. (1973). Pharmacology of fusaric acid in man. *Clin. Pharmacol. Ther.*, **14**, 541–546.

MULLER, B.D. & BELL, C. (1986). Vesicular storage of 3,4-dihydroxyphenylamine and noradrenaline in terminal sympathetic nerves of dog spleen and kidney. *J. Neurochem.*, **47**, 1370–1375.

NEUMAN, B., WIEDERMANN, C.J., FISHER-COLBRIE, R., SCHOBER, M., SPERK, G. & WINKLER, H. (1984). Biochemical and functional properties of small and large dense-core vesicles in sympathetic nerves of rat and ox vas deferens. *Neuroscience*, **13**, 921–931.

OHLSTEIN, E.H., KREUSE, L.I., EZEKIEL, M., SHERMAN, S.S., ERICKSON, R., DEWOLF, W.E. & BERKOWITZ, B.A. (1987). Cardiovascular effects of a new potent dopamine β-hydroxylase inhibitor in spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.*, **241**, 554–559.

SOARES-DA-SILVA, P. (1986). Evidence for a non-precursor dopamine pool in noradrenergic neurones of the dog mesenteric artery. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **333**, 219–223.

SOARES-DA-SILVA, P. (1987a). A comparison between the pattern of dopamine and noradrenaline release from sympathetic neurones of the dog mesenteric artery. *Br. J. Pharmacol.*, **90**, 91–98.

SOARES-DA-SILVA, P. (1987b). Dopamine released from nerve terminals activates prejunctional dopamine receptors in dog mesenteric arterial vessels. *Br. J. Pharmacol.*, **91**, 591–599.

SOARES-DA-SILVA, P. (1988). Dopamine and noradrenergic transmission. In *Mechanisms of Vasodilatation*. ed. Vanhoutte, P.M. New York: Raven Press, (in press).

SOARES-DA-SILVA, P. & AZEVEDO, I. (1985). Fibroblasts and sympathetic innervation of blood vessels. *Blood Vessels*, **22**, 278–285.

SOARES-DA-SILVA, P. & AZEVEDO, I. (1988). Differential effects of 6-hydroxydopamine on the two types of nerve vesicles and dopamine and noradrenaline content in mesenteric arterial vessels. *J. Auton. Pharmacol.*, (in press).

SOARES-DA-SILVA, P. & DAVIDSON, R. (1985). Effects of 6-hydroxydopamine on dopamine and noradrenaline contents in dog blood vessels and heart: Evidence for a noradrenaline-independent dopamine pool. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **329**, 253–257.

STARKE, K. (1987). Presynaptic α-autoreceptors. *Rev. Physiol. Biochem. Pharmacol.*, **107**, 73–146.

WILLEMS, J.L., BUYLAERT, W.A., LEFEVBRE, R.A. & BOGAERT, M. (1985). Neuronal dopamine receptors on autonomic ganglia and sympathetic nerves and dopamine receptors in the gastrointestinal system. *Pharmacol. Rev.*, **37**, 165–215.

(Received March 8, 1988
Revised April 19, 1988
Accepted May 9, 1988)