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Effects of L-Dopa and L-Tyrosine on Adrenergic Transmission in the Canine Saphenous Vein[1] (41440)

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Abstract. The effects of L-dopa, and L-tyrosine on norepinephrine (NE) and dopamine (DA) content and overflow from adrenergically innervated canine saphenous veins were determined. L-Dopa treatment resulted in large increases in concentration of DA in tissue, and caused significant amounts of DA to overflow into the superfusate both under basal conditions and during electrical stimulation. The DA did not appear to be of vesicular origin, since DA concentrations in superfusate were not altered by removing Ca^{2+} from the medium. The increased amounts of DA in the junctional cleft did not appear to activate prejunctional dopaminergic or α-adrenergic receptors, since endogenous NE overflow in treated veins was not lower than that observed in untreated veins. Evoked tension responses were also not changed in L-dopa-treated veins indicating that DA did not activate postsynaptic α- or β-adrenergic receptors. Treatment with L-dopa, but not with L-tyrosine, caused small increases in tissue content of NE. Treatment with $5 \times 10^{-5}M$ L-dopa or $5 \times 10^{-4}M$ L-tyrosine attenuated the decrease in NE overflow which was found consistently in untreated veins. However, factors other than lack of precursor must have been responsible for the decrease in NE overflow, since release of NE (measured in the presence of blockers of neuronal and extraneuronal uptakes) did not decrease upon repeated nerve stimulations.

L-Dopa is used widely to treat patients with Parkinson's disease (1–3). Patients only very rarely develop hypertension during L-dopa therapy, although L-dopa is a precursor of L-norepinephrine (L-NE), a potent vasoconstrictor substance. Paradoxically, hypotension is a rather frequent side effect seen during L-dopa treatment (1, 2). The hypotensive effects of L-dopa treatment are thought to be mediated by central mechanisms (4), but as pointed out by Westfall (5), effects at the level of the peripheral adrenergic nerve terminal have not yet been studied and may contribute to the vasodilator effects of L-dopa.

In the present experiments we have studied the effects of L-dopa on basal and evoked release of catecholamines at a peripheral neuroeffector junction. The concentration of L-dopa that was used (10 μg/ml, 5×10^{-5} *M*) was one that approximates that found in plasma of patients during L-dopa treatment (1 to 8 μg/ml) (3). *In vitro,* superfused, helically cut strips of dog saphenous vein were used in the study because this vessel has been shown to be richly innervated and, in addition, many of the characteristics of transmitter release and disposition in this vessel have been described (6–8). Experiments were also done to determine the effects of two concentrations (5×10^{-5} *M* and 5×10^{-4} *M*) of tyrosine, the remote precursor of NE, on basal and evoked release of catecholamines in this preparation. A preliminary presentation of some of this data has been made (9).

Methods. *Preparation and superfusion of the veins.* The experiments were performed on saphenous veins taken from dogs (15 to 25 kg) anesthetized with pentobarbital (30 mg/kg iv). The veins were cleaned of perivascular tissue and mounted for superfusion at 37°. The veins ranged in weight from 80 to 150 mg with a mean of 120 mg. The superfusing fluid was Krebs–Ringer bicarbonate solution of the following millimolar composition: NaCl, 118.1; KCl, 4.7;

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$MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11.1; and disodium ethylenediaminetetraacetate (EDTA), 0.03. Sodium metabisulfite (1 g/liter) was added as an antioxidant. The veins were superfused at 1.5 ml/min. The solution was aerated continuously with a mixture of 95% O_2 and 5% CO_2. Isometric tension was recorded continuously.

For stimulation of adrenergic nerve endings in the preparations, two platinum electrodes were placed parallel to, and in contact with, the veins. Electrical impulses consisted of rectangular waves (9 V, 2 msec, 5 Hz.) provided by a direct-current power supply and a switching transistor triggered by a Grass stimulator (Model S44). The veins were stimulated continuously for three (T_1, T_2, and T_3) 20-min periods. Thirty-minute intervals were allowed between each period of stimulation.

Measurement of NE concentration from vein tissue and superfusate. Following stimulation, the veins were blotted lightly and weighed, and NE and DA were extracted by placing the tissue for two 30-min periods in separate 2.5-ml aliquots of 1 *N* acetic acid containing 0.03 m*M* EDTA and 0.2 mg/ml ascorbic acid. A comparison of this method for NE and DA extraction has previously been made with extraction involving homogenization of the tissue in 2 *N* acetic acid. The amounts of NE and DA obtained from paired saphenous vein strips from the same dog using the former method were not significantly different from values obtained using the latter method (6).

NE and dopamine (DA) were isolated from the vein extracts and from the superfusate by adsorption chromatography on alumina and cation exchange resin as previously described (6, 10). This method involves the concentration of relatively large volumes of superfusate (30 ml) collected over 20 min into 1 ml of 0.67 *M* boric acid. The concentration of NE and DA in a 25-μl aliquot of this extract was determined by liquid chromatography with electrochemical detection (LCEC) using a reversed phase separation (a C_{18} μBondapak column, and a mobile phase of 0.07 *M* NaH_2PO_4, 0.2 m*M* disodium EDTA, 2 m*M* heptanesulfonate, and 0.8% acetonitrile at pH 4.8). An electrochemical detector with an applied potential of 0.65 V against a silver/silver chloride reference electrode was used. The limit of sensitivity of this method (defined by a signal-to-noise ratio greater than 5) was 25 pg for NE, and 40 pg for DA injected on the column. The average recoveries of NE and DA (10 ng added to 30 to 50 ml of the superfusate) were 74.1 ± 1.9 and 68.9 ± 2.7% respectively in 20 extractions. Data are corrected for these recoveries.

Calculation of amine overflow as percentage of tissue content. Release of endogenous NE and DA during basal conditions or during electrical stimulation was expressed as percentage of tissue amine content that had been released during 20 min of superfusion. The amount of amine present in the tissue at the start of any time interval was calculated by adding cumulatively the amounts of amine released in each superfusate fraction to the amount present in the saphenous vein at the end of the experiment.

Drugs used. The following compounds were used: L-dopa (5×10^{-5} *M*); L-tyrosine (5×10^{-5} and 5×10^{-4} *M*); cocaine hydrochloride (1×10^{-5} *M*) and corticosterone (2×10^{-4} *M*). The latter two drugs were added to the Krebs–Ringer solution used for superfusion. In the L-dopa and L-tyrosine experiments the veins were both preincubated for 15 min, and superfused for 240 min with Krebs–Ringer solution containing L-dopa and L-tyrosine.

Results. *Chromatographic separations.* NE, DA, and L-dopa were clearly separated by the chromatographic separation used. Retention times were: 3 min for L-dopa, 6 min for NE, and 21 min for DA. L-Dopa was effectively removed from superfusate by the two-column extraction procedure. NE and DA were identified in aliquots of the boric acid eluate prepared from samples of superfusate by comparing their retention times in the LCEC separations with those of authentic NE and DA. We have previously shown an excellent correlation between the amounts of NE measured by this method and those measured by a fluorometric procedure (6).

NE and DA concentrations in saphenous

veins. The tissue contents of NE and DA in vein segments which had just been removed from the dogs (without superfusion, stimulation, or treatment with any amino acid precursors) were 3.65 μg/g tissue and 0.06 μg/g tissue, respectively (Table I). The tissue contents of catecholamines in segments of control veins after superfusion (240 min duration, during which there were three 20-min periods of electrical stimulation) were 3.09 μg NE/g tissue and 0.06 μg DA/g tissue. These values were not different from those in the unstimulated veins. In vein segments which had been treated with L-dopa for 15 min before superfusion and during the 240 min of superfusion (including the stimulations periods) DA concentrations were 11-fold higher, and NE concentrations were 1.5-fold higher, than in the untreated veins after superfusion and stimulation (Table I). Similar treatments with L-tyrosine (5×10^{-5} *M* or 5×10^{-4} *M*) did not result in changes in the concentrations of either NE or DA in the veins (Table I).

NE and DA in superfusate. The overflow of NE from veins under basal, unstimulated conditions could not be measured reproducibly because the amounts overflowing were close to, or below, the sensitivity of the method. In preparations where basal efflux of NE in superfusate could be quantified, it decreased slightly with time and was progressively less in the second and third basal periods (Table II).

TABLE I. CONCENTRATION OF NE AND DA IN SAPHENOUS VEINS: EFFECTS OF L-DOPA AND L-TYROSINE

Treatment	Amine concentration (μg/g)	
	NE	DA
Control, no superfusion	3.65 ± 0.41	0.06 ± 0.02
Control, after superfusion[a]	3.09 ± 0.24	0.06 ± 0.02
L-Dopa, 5×10^{-5} *M*[a,b]	4.42 ± 0.47*	0.70 ± 0.13*
L-Tyrosine, 5×10^{-5} *M*[a,b]	3.23 ± 0.35	0.08 ± 0.01
L-Tyrosine, 5×10^{-4} *M*[a,b]	3.27 ± 0.22	0.08 ± 0.01

[a] Veins had been superfused for 240 min. Electrical stimulation (5 Hz) was applied between 120 and 140 min, between 170 and 190 min, and between 220 and 240 min of superfusion.

[b] Treatment with L-dopa or L-tyrosine entailed both preincubation for 15 min and superfusion of the tissue with Krebs–Ringer solution containing the amino acid.

* Significantly different from control veins after superfusion: Student's *t* test for unpaired data ($P < 0.05$).

Electrical stimulation consistently released NE into superfusate in amounts which could be measured easily by the LCEC procedure (Table II). The amounts of NE released into superfusate in the first period of stimulation averaged 8.9 fmole/mg tissue/min, which was equivalent to a release of 1.0% of total tissue content in a 20-min period. In each of seven veins the amount of NE in superfusate decreased during the second and third periods of electrical stimulation; this decrease was significant by the ranked sign test ($P < 0.05$).

L-Dopa treatment did not result in an increased basal overflow of NE from unstimulated veins. The overflow of NE from L-dopa-treated veins during the first period of stimulation was not different from controls; however, during the second and third stimulations significantly more NE overflowed from the L-dopa-treated veins than from the controls (Table II). Thus the decrease in overflow of NE during repeated stimulations was attenuated by the L-dopa treatment. The percentage of tissue NE that overflowed during stimulation was, however, no different in control veins and veins which were superfused with L-dopa.

DA was not detected in superfusate of control veins either under basal conditions or during nerve stimulation (Table II). Overflow of DA was observed during basal conditions in the L-dopa-treated veins (Table II). During stimulation, DA overflow from the L-dopa-treated veins increased approximately twofold with the result that the amounts of DA in superfusate equalled, or sometimes exceeded, the amounts of NE that were present (Table II and Fig. 1). The amounts of DA in superfusate in the L-dopa-treated veins varied widely, both under basal conditions and during nerve stimulation. The percentage of tissue amine that was released during each stimulation period was significantly greater ($P < 0.05$) for DA than for NE in the veins treated with L-dopa (Table II).

Treatment with 5×10^{-5} *M* L-tyrosine did not cause significant overflow of NE under basal conditions, nor did it cause changes in the absolute amount of NE or the percentage of tissue NE that overflowed during electrical stimulation (Table III). Treatment

TABLE II. EFFECTS OF L-DOPA ON BASAL AND EVOKED RELEASE OF NOREPINEPHRINE (NE) AND DOPAMINE (DA) FROM CANINE SAPHENOUS VEINS

Time period	Control veins[b]		Veins treated with L-dopa (5×10^{-5} M)	
	NE	DA	NE	DA
	(A) Absolute release[a]			
Basal 1	1.7 ± 0.8	N.D.[c]	1.9 ± 1.5	10.1 ± 6.5*
Stimulation 1	8.9 ± 2.2	N.D.	11.4 ± 1.6	23.5 ± 10.2*
Basal 2	1.0 ± 0.9	N.D.	3.1 ± 1.0	13.9 ± 10.6*
Stimulation 2	6.1 ± 1.3	N.D.	10.1 ± 0.8*	18.3 ± 7.8*
Basal 3	0.6 ± 0.4	N.D.	1.4 ± 0.9	9.4 ± 6.6*
Stimulation 3	4.7 ± 1.2[d]	N.D.	8.8 ± 0.9*	18.2 ± 10.1*
	(B) Release as percentage of tissue content[e]			
Basal 1	0.1 ± 0.1	N.D.	0.1 ± 0.1	3.1 ± 2.1*
Stimulation 1	1.0 ± 0.3	N.D.	0.9 ± 0.2	10.1 ± 3.9*
Basal 2	0.1 ± 0.1	N.D.	0.3 ± 0.1	3.9 ± 2.4*
Stimulation 2	0.7 ± 0.1	N.D.	0.8 ± 0.1	8.9 ± 3.0*
Basal 3	0.1 ± 0.0	N.D.	0.1 ± 0.0	3.0 ± 1.9*
Stimulation 3	0.5 ± 0.1	N.D.	0.8 ± 0.2	9.4 ± 3.9*

[a] Data presented as fmole NE or DA/mg tissue/min.
[b] Data as means ± SEM of seven determinations.
[c] The amount of DA in the sample is not detectable (N.D.) with this method.
[d] Significantly different from values obtained with stimulation 1: ranked sign test.
[e] Data as percentage of tissue NE which was released per 20-min time period.
* Significantly different from control veins ($P < 0.05$).

with 5×10^{-4} M L-tyrosine did not affect basal release of NE but resulted in significant increases over control both in absolute release of NE, and percentage of tissue NE that was released during the second and third periods of electrical stimulation. Thus, the decrease in evoked release of NE with repeated stimulation was attenuated in veins treated with the higher concentration of L-tyrosine (Table III). DA was not detected in superfusates of veins treated with L-tyrosine, either during basal conditions or during nerve stimulation.

Effect of Ca^{2+} on overflow of DA. To determine whether DA overflowing into the superfusate of L-dopa-treated veins originated in the vesicular or nonvesicular compartment of the neuron, the effect of removing Ca^{2+} from the incubation medium and superfusate was examined. In the veins treated with L-dopa and superfused with Krebs–Ringer solution without Ca^{2+} (containing 1 mM [ethylenebis(oxyethylenenitrilo)]-tetraacetic acid (EGTA)) DA was detected in the superfusate both during basal conditions and during electrical stimulation. The values obtained were not significantly different from those obtained from veins superfused with Krebs–Ringer solution containing normal Ca^{2+} (Table IV). In contrast, NE was released during electrical stimulation in the presence of Ca^{2+}, but was never detected in the superfusate in the absence of Ca^{2+} (Table IV).

Effects of cocaine and corticosterone on NE concentrations in superfusate. In the presence of the neuronal and extraneuronal uptake blockers cocaine (10^{-5} M) and corticosterone (2×10^{-4} M), the overflow of NE evoked by nerve stimulation was increased approximately 3.5-fold (Table V). There were no decreases in NE release in the second and third periods of stimulation in cocaine- and corticosterone-treated veins.

Isometric tension developed in control and treated veins. In seven control veins increases in tension were obtained by stimulation at 5 Hz for 20 min. The contractile response was maximal at 3 min and remained stable throughout the period of stimulation. In those control veins the tensions that developed during first, second, and third periods of electrical stimulation were 3.07 ± 0.29, 2.90 ± 0.28, and 2.87 ±

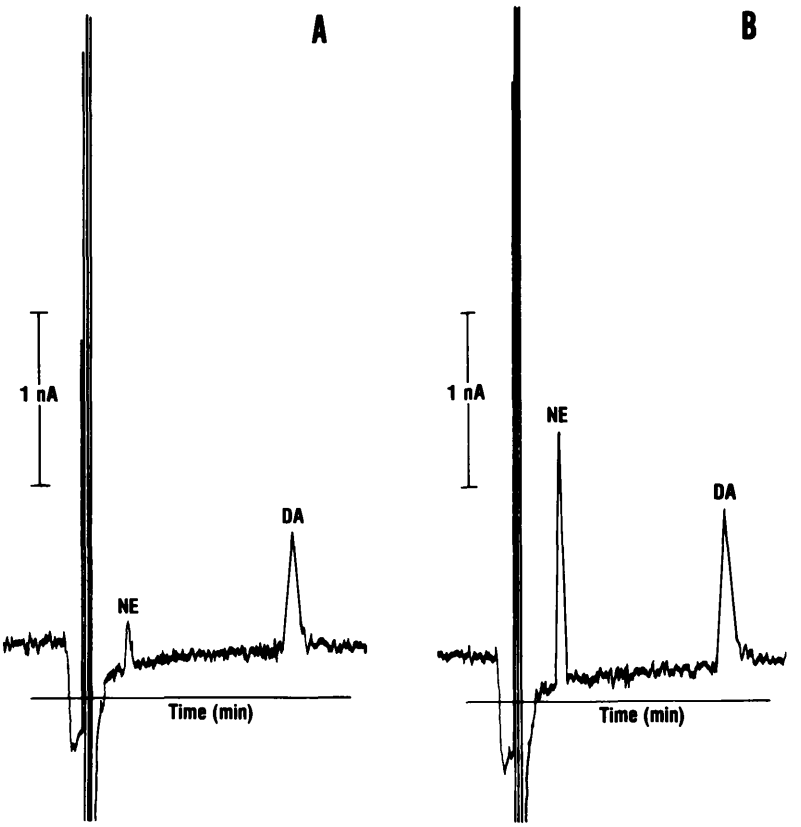

FIG. 1. Chromatogram generated by injection of 25 μl of boric acid eluate obtained after concentrating 30 ml of Krebs–Ringer solution collected (A) during 20 min of basal efflux and (B) during 20 min of electrical stimulation. Both veins were superfused with Krebs–Ringer solution containing L-dopa (5×10^{-5} *M*). Elution time for NE was 6 min and for DA 21 min.

0.22 g, respectively. The contractile tensions obtained in veins treated with L-dopa were 2.84 ± 0.26, 2.88 ± 0.32, and 2.88 ± 0.34 g. In veins treated with L-tyrosine the contractile responses developed during the three periods of electrical stimulation were 3.10 ± 0.40, 3.00 ± 0.37, and 2.90 ± 0.37 g with 5×10^{-5} *M* tyrosine, and 3.38 ± 0.35, 3.50 ± 0.46, and 3.50 ± 0.53 g with 5×10^{-4} *M* tyrosine. The values obtained in the presence of L-dopa and tyrosine were not significantly different from those obtained in untreated veins.

Discussion. The present studies showed that L-dopa treatment increased the concentration of DA in canine saphenous veins. In addition, considerable amounts of DA were released from L-dopa-treated veins, both under basal conditions, and during nerve stimulation. This DA did not appear to be vesicular in origin, since DA concentrations in superfusate were not altered by removing Ca^{2+} from the medium. Entry of Ca^{2+} ions is considered to be an essential step for release of vesicular contents by exocytosis (11). DA was never detected in superfusates from control veins, although the sensitivity of the method is such that amounts as low as 3.5 fmol/mg vein/min could be detected.

The amounts of DA present in superfusates of L-dopa-treated veins were highly variable, although our recoveries of added DA were consistent and reproducible. DA in neuroplasm is subject to uptake into NE storage vesicles, and to metabolism by monoamine oxidase (MAO), or it can overflow into the junctional cleft. MAO activity,

TABLE III. EFFECTS OF L-TYROSINE ON BASAL AND EVOKED RELEASE OF NOREPINEPHRINE FROM CANINE SAPHENOUS VEINS[a]

Time period	Control veins	Veins treated with L-tyrosine	
		5×10^{-5} M	5×10^{-4} M
	(A) Absolute release[b]		
Basal 1	1.3 ± 0.7	N.D.	N.D.
Stimulation 1	8.8 ± 1.6	8.8 ± 1.2	11.5 ± 1.0
Basal 2	0.8 ± 0.6	N.D.	N.D.
Stimulation 2	6.1 ± 1.1[d]	6.9 ± 0.8	11.3 ± 1.0*,**
Basal 3	0.4 ± 0.3	N.D.	N.D.
Stimulation 3	4.6 ± 0.9[d]	7.1 ± 2.2	9.7 ± 1.5*
	(B) Release as percentage of tissue content[c]		
Basal 1	0.1 ± 0.1	N.D.	N.D.
Stimulation 1	1.0 ± 0.2	0.9 ± 0.1	1.2 ± 0.2
Basal 2	0.1 ± 0.1	N.D.	N.D.
Stimulation 2	0.7 ± 0.1[d]	0.8 ± 0.1	1.2 ± 0.2*
Basal 3	0.0 ± 0.0	N.D.	N.D.
Stimulation 3	0.5 ± 0.1[d]	0.8 ± 0.2	1.0 ± 0.2*

[a] Data as means ± SEM of seven determinations.
[b] Data as fmole NE/mg tissue/min.
[c] Data release in 20-min period as a percentage of tissue content.
[d] Significantly different from stimulation 1.
* Significantly different from control veins ($P < 0.05$).
** Significantly different from veins treated with 5×10^{-5} M tyrosine ($P < 0.05$).

and catecholamine uptake and storage mechanisms are known to change markedly during development and aging (12). The wide variation in DA outflow observed in our study might have resulted because dogs of different ages were used in the study.

L-Dopa treatment also resulted in higher concentrations of NE present in the veins following stimulation. The increase in concentration for NE is about the same magnitude as for DA, but appeared less striking in relation to the concentration of amine in the untreated veins.

The high levels of DA present in superfusates from L-dopa-treated veins had no discernable effects on basal overflow of NE from veins. It has previously been shown in dog saphenous vein (13) that high concentrations of exogenous DA may displace NE from its storage sites, an action which is blocked by cocaine (14). The high levels of DA present in superfusates from L-dopa-treated veins also did not greatly affect the amounts of NE released from tissue during nerve stimulation. Apart from a stimulation of NE synthesis which might be expected due to increased availability of the immediate precursor, an inhibition of NE release might also have been expected since it has been shown that high concentrations of exogenous DA inhibit NE release via an effect on presynaptic α receptors (15), or on specific presynaptic dopaminergic receptors (5, 16–18). The action of DA on NE release and NE synthesis would have opposite effects on the amounts of NE in the cleft, and if these actions were operative in the present studies, they must cancel each other out, since the net result was that NE overflow from the L-dopa-treated veins was little changed from that in controls (apart from an attenuation of the decrease in NE overflow characteristic of repeated stimulations).

Under our experimental conditions α_2 adrenoreceptors are probably not maximally activated during electrical stimulation.

TABLE IV. BASAL AND EVOKED RELEASE OF NE AND DA FROM VEINS TREATED WITH L-DOPA IN THE PRESENCE AND ABSENCE OF CALCIUM[a]

Time period	NE release (fmole/mg tissue/min)		DA release (fmole/mg tissue/min)	
	Ca^{2+} 2.5 mM	Ca^{2+} absent	Ca^{2+} 2.5 mM	Ca^{2+} absent
Basal 1	N.D.[b]	N.D.	39.3 ± 35.4	22.3 ± 10.3
Stimulation 1	15.8 ± 1.8	N.D.	62.4 ± 39.1	47.7 ± 14.4
Basal 2	N.D.	N.D.	57.7 ± 33.8	20.9 ± 9.4
Stimulation 2	14.7 ± 1.6	N.D.	68.9 ± 28.1	26.9 ± 10.9
Basal 3	N.D.	N.D.	53.6 ± 16.6	10.9 ± 2.2[c]
Stimulation 3	10.4 ± 0.7	N.D.	74.9 ± 23.9	33.5 ± 7.9

[a] Data as means ± SEM of four determinations.
[b] Not detected.
[c] Significant difference from veins superfused with Ca^{2+} 2.5 mM.

TABLE V. COMPARISON OF EVOKED NOREPINEPHRINE RELEASE BETWEEN UNTREATED VEINS AND VEINS TREATED WITH BOTH COCAINE (10^{-5} *M*) AND CORTICOSTERONE (2×10^{-4} *M*) DURING THREE PERIODS OF ELECTRICAL STIMULATION

	NE fmole/mg/min	
	Untreated veins	Treated veins
Stimulation 1	9.3 ± 1.9	31.2 ± 2.9[b]
Stimulation 2	6.8 ± 1.3	33.8 ± 3.7[b]
Stimulation 3	5.3 ± 1.1[a]	32.7 ± 3.9[b]

[a] Significantly different from values obtained with stimulation 1.

[b] Significantly different from values obtained in untreated veins.

Under similar experimental conditions, Lorenz *et al.* (19) and Hyatt *et al.* (20) have demonstrated that when the α_2 agonist, NE, was infused following 14 to 16 min of electrical stimulation, [^{3}H]NE release was inhibited, a presynaptic α receptor-mediated effect. Although presynaptic α effects have been demonstrated to occur under these conditions, they may not have been observed in the present experiments because DA formed from L-dopa may not have the same access to presynaptic receptors as NE.

The high levels of DA present in superfusates of the L-dopa-treated veins appeared to have no effects on the responses of effector cells to sympathetic nerve stimulation since there were no differences between the tensions which developed in control veins and in L-dopa-treated veins. DA has previously been shown to depress responses of effector cells to sympathetic nerve stimulation in several preparations (17, 18, 21). On the other hand high concentrations of DA (10^{-5} *M*) have been shown to potentiate responses to nerve stimulation in the rabbit mesoduodenal artery (22).

Responses to DA in the present study appeared to be different in several respects from those observed previously in isolated vascular preparations. This apparent discrepancy may reflect differences in the responses to exogenous and to endogenous DA. In addition, previous studies have usually utilized much higher concentrations of DA than those present in superfusates in our studies. While it would have been desirable to determine the effects of different concentrations of L-dopa, the concentration chosen (5×10^{-5} *M*) resulted in concentrations of DA in superfusate values which closely approximated those in plasma (1–2 ng/ml) of patients with Parkinsons disease (23).

Thus, the present studies indicate that in dog saphenous veins, treatment with L-dopa resulted in the overflow of considerable amounts of DA into the junctional cleft but that this DA has minimal effects on NE release or on the action of NE at postjunctional sites. Thus we can show no effect of L-dopa on this peripheral neuroeffector junction which might account for the hypotensive effects seen when L-dopa is given to patients. It is, however, possible that other vessels may be more sensitive to DA; in these vessels pertubations of NE release or action might result which could contribute to the vasodilator effects of L-dopa.

L-Tyrosine treatment did not alter the content of NE or of DA in veins. However, the amounts of NE that were released during electrical stimulation were significantly increased by higher concentrations of the amino acid (Table III). Recently it has been shown that tyrosine administration enhances the synthesis of NE in rat brain, suggesting that tyrosine hydroxylase is not saturated at normal concentrations of tyrosine in tissue (24, 25).

We have been interested in the mechanism responsible for the decreased release of NE during repeated periods of nerve stimulation. Previously we have shown that the decrease was not caused by stimulation of presynaptic receptors, since blockade of these receptors with phenoxybenzamine still resulted in decreases of NE overflow (26). Accordingly, we considered that this decrease might be due to decreased availability of precursors of NE. The present experiments suggest that this may indeed be a factor because when the content of NE in the vein was increased by administration of L-dopa or high concentrations of tyrosine, the decrease in NE release during repeated stimulation was attenuated (Table

III). However, other factors may also be involved because in veins NE release (measured in the presence of uptake blockers) did not decrease during repeated stimulations of veins (Table V), suggesting that this decrease in overflow during prolonged nerve stimulation may result from facilitated removal of NE from the junctional cleft due to enhancement of neuronal or extraneuronal uptake of NE or an activation of MAO or of catechol *O*-methyltransferase (27).

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