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In vivo activity of tyrosine hydroxylase in rat adrenal glands following administration of quinpirole and dopamine

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Abstract

Using adrenal dopamine as indicator we have previously obtained evidence that quinpirole and several other agonists on dopamine D_2-like receptors acutely stimulate the synthesis of adrenal catecholamines. In the present study we measured the effect of quinpirole and dopamine on the hydroxylation of tyrosine in the adrenals, using the method of DOPA (3,4-dihydroxyphenylalanine) accumulation following the administration of the inhibitor of aromatic L-amino acid decarboxylase NSD 1015 (3-hydroxybenzylhydrazyne). In view of the large amounts of catecholamines in the adrenal tissue samples, this necessitated a modification of the method for analysing DOPA. Both quinpirole and dopamine significantly enhanced the rate of DOPA accumulation in the adrenals, indicating stimulation of adrenal tyrosine hydroxylase. The effect of dopamine was blocked by domperidone, a dopamine D_2 receptor antagonist that penetrates poorly into the central nervous system. Thus the effect of dopamine, which itself penetrates poorly into the central nervous system, was presumably mediated peripherally. Similarly epinine, i.e. the *N*-methyl derivative of dopamine, appeared to enhance adrenal catecholamine synthesis, as indicated by an elevated adrenal dopamine level. The data support the view that stimulation of peripherally located dopamine D_2-like receptors can enhance the rate of adrenal catecholamine synthesis by stimulating the activity of tyrosine hydroxylase.

Keywords: Dopamine; Dopamine receptor agonist; Catecholamine, synthesis; DOPA (3,4-dihydroxyphenylalanine), accumulation; Adrenal medulla

1. Introduction

We have previously reported on an acute elevation of adrenal dopamine levels induced by quinpirole and other dopamine D_2-like receptor agonists (Kujacic et al., 1990, 1991). Moreover, we have presented evidence supporting the view that this effect is a consequence of increased catecholamine synthesis (Kujacic and Carlsson, 1993). In order to further investigate the mechanism involved, we have now extended the study to include the first, rate-limiting step in the synthesis of catecholamines, i.e. the hydroxylation of tyrosine by tyrosine hydroxylase to form DOPA (3,4-dihydroxyphenylalanine).

The rate of tyrosine hydroxylation was measured by determining the rate of DOPA accumulation following inhibition of the aromatic L-amino acid decarboxylase by means of NSD 1015 (3-hydroxybenzylhydrazyne, Carlsson et al., 1972; Kehr et al., 1972). To adapt this technique to the adrenal medulla necessitated a modification of the analytical method because of the high levels of adrenaline and noradrenaline in the tissue samples. The use of this technique permitted us to measure the effects on adrenal catecholamine synthesis not only of exogenous dopamine receptor agonists but also of dopamine itself. For comparison we also studied the effect of epinine, i.e. the *N*-methyl derivative of dopamine, on the adrenal dopamine level. Epinine has been shown to possess a high affinity for dopamine D_1 and D_2 receptors (Casagrande et al., 1989; Itoh, 1991).

2. Materials and methods

2.1. Animals

The experiments were performed in daylight hours on male albino rats of the Sprague-Dawley strain

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(ALAB, Solletuna, Sweden), weighing 200–300 g, not earlier than one week after they were received from the supplier. The animals were kept five per cage under controlled environmental conditions. Standard laboratory chow and tap water were allowed ad libitum.

2.2. Drugs

The following drugs were administered: quinpirole hydrochloride (LY 171555) (Eli Lilly and Co., Indianapolis, IN, USA), dopamine hydrochloride (Sigma, St. Louis, MO, USA), epinine hydrochloride (deoxyepinephrine; *N*-methyldopamine) (Sigma, St. Louis, MO, USA), domperidone (Janssen Pharmaceutica, Beerse, Belgium) and NSD 1015 (3-hydroxybenzylhydrazyne dihydrochloride) (Fluka Chemie, Switzerland).

Quinpirole, dopamine and epinine were dissolved in physiological saline (0.9% NaCl), NSD 1015 in distilled water, and domperidone in minimal quantities of glacial acetic acid and diluted with 5.5% warm glucose solution.

Control groups were injected with physiological saline (0.9% NaCl), or NSD 1015, respectively, as indicated.

The compounds were injected subcutaneously into the neck region in a volume of 5 ml/kg body weight, except NSD 1015, which was injected in a volume of 10 ml/kg body weight.

2.3. Dissection and determination of monoamines

The rats were decapitated after various periods of drug administration and adrenal glands, hearts and forebrains were rapidly removed, dissected and immediately frozen on dry ice. Tissue parts were weighed and stored at −70° C until analysis.

Tissue catecholamine levels were determined by means of high performance liquid chromatography with electrochemical detection (HPLC-EC) according to standard principles (Kujacic et al., 1990).

2.4. Determination of DOPA in the adrenal glands

When the adrenal extract was purified on alumina, the large amounts of adrenaline and noradrenaline in the adrenal glands interfered with the HPLC-EC assay of DOPA and the problem could not be eliminated by, e.g., changing the composition of the mobile phase. It was thus judged necessary to remove the catecholamines from the tissue extract before the HPLC procedure. This was done by passing the extract through a cation exchange column at neutral pH, thereby trapping the catecholamines, but letting DOPA through the column. The recovery of DOPA after Dowex extraction was almost 100% (Atack and Magnusson, 1978). The procedure was carried out as follows: Each pair of adrenal glands was homogenised in 3 ml of 0.1 M perchloric acid, containing 50 μl of 10% sodium EDTA and 50 μl of 5% gluthatione. After centrifugation (10000 × *g*, 10 min) followed by filtration, the pH of the supernatant was adjusted to 6 by adding 150 μl of 1 M K_2CO_3. After the mixture was cooled in an ice bath for 20 min, $KClO_4$ precipitated. 2 ml of the supernatant was put on a column of Dowex 50W, X-4, 200–400 mesh (resin bed 4.0 mm diameter, 75 mm length, in sodium form; for details on the preparation of the resin and the column, see Atack and Magnusson, 1978). The effluent and the first 3.0 ml fraction of 0.1 M phosphate buffer (pH 6.5) were collected. The combined effluent was further prepared and analysed by means of HPLC-EC according to the principles previously described (Kujacic et al., 1990). The mobile phase contained 0.015 M K_2HPO_4, 0.035 M citric acid · H_2O, 0.26 mM sodium octylsulphate, 0.054 mM sodium EDTA and 3% v/v of methanol. pH was adjusted to 2.5.

A somewhat similar method, albeit using a weak rather than strong cation exchange resin for primary purification, has been reported by Hayashi et al. (1988).

2.5. Statistics

Fisher's PLSD (protected least significant difference) test preceded by one-way analysis of variance (ANOVA) was applied (Milliken and Johnson, 1984). Probability levels of less than 0.05 were regarded as statistically significant.

3. Results

3.1. DOPA determination after aromatic L-amino acid decarboxylase inhibition by NSD 1015 in adrenal glands

After purification of the samples by alumina absorption alone, a method usually used for the purification of catecholamines and DOPA, large peaks of adrenaline and noradrenaline interfered with the accurate determination of DOPA by HPLC-EC. Changing the pH of the mobile phase and consequently increasing the retention time of DOPA (maximal at pH 2.5), without changing the other chromatographic conditions, seemed to be the easiest method, but was not effective enough to separate DOPA from adrenaline in the adrenal glands (see Fig. 1; the arrow indicates the retention time of DOPA). In contrast, after purification of samples on single columns of a strongly acidic cation-exchange resin, Dowex 50W, followed by alumina absorption, noradrenaline and adrenaline were almost completely eliminated and the peak of DOPA was clearly separated (Fig. 2).

3.2. Quinpirole-induced effects after aromatic L-amino acid decarboxylase inhibition by NSD 1015

Inhibition of catecholamine synthesis by NSD 1015 (100 mg/kg s.c., 30 min) decreased adrenal dopamine levels by about 45% compared to saline-treated controls and also completely blocked the significant increase in adrenal dopamine induced by quinpirole (1 mg/kg s.c., 35 min) (Fig. 3A). However, no NSD 1015-induced effects were observed on the adrenaline content in the heart, and the increase in heart adrenaline induced by quinpirole was not blocked by NSD 1015 (Fig. 3A). This increase is probably caused by adrenaline release from the adrenal medulla (Kujacic et al., 1995).

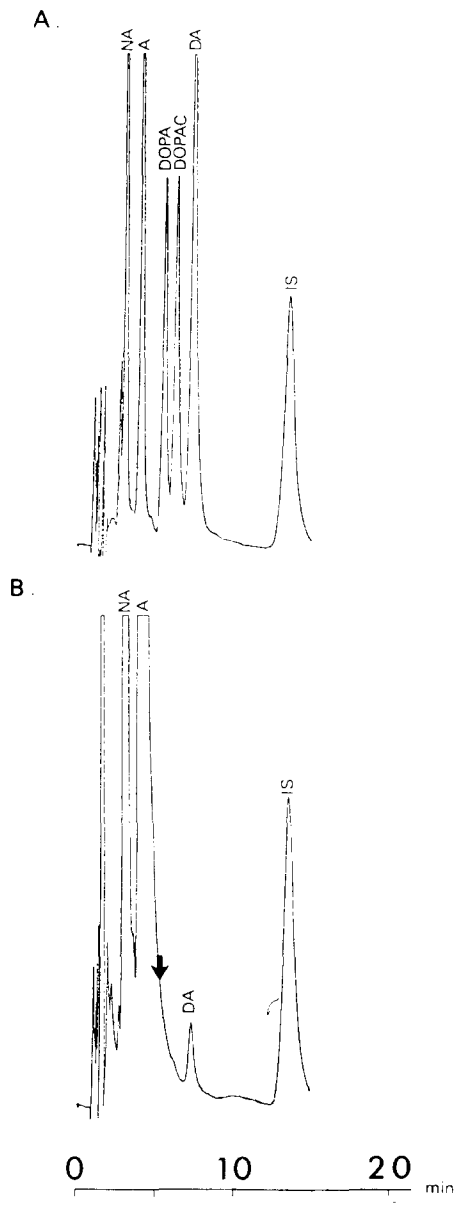

Fig. 1. A: Chromatogram of a catechol standard sample. Chromatographic conditions: mobile phase – pH 2.5, 7% methanol, 60 mg/l sodium octylsulphate; flow rate 1.5 ml/min; electrode potential +0.70 V. Internal standard (IS): α-methyl-DOPA. B: Chromatogram of extract of rat adrenal glands (NSD 1015-treated controls). Samples were purified by alumina absorption alone. Chromatographic conditions were the same as for the standard sample. The arrow indicates the retention time of DOPA.

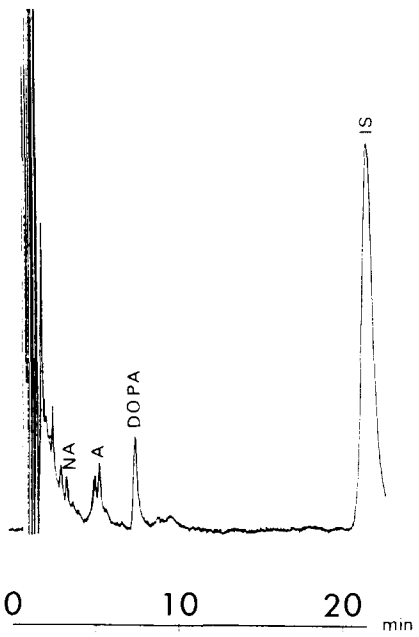

Fig. 2. Chromatogram of extract of rat adrenal glands (NSD 1015-treated controls). Samples were purified on single columns of a strongly acidic cation-exchange resin, Dowex 50W, X-4 (200–400 mesh), followed by alumina absorption. Chromatographic conditions: mobile phase – pH 2.5, 3% methanol, 60 mg/l sodium octylsulphate; flow rate 1.5 ml/min; electrode potential +0.70 V. Internal standard (IS): α-methyl-DOPA.

Quinpirole (1 mg/kg, 35 min) significantly enhanced the DOPA accumulation induced by NSD 1015 (100 mg/kg, 30 min) by about 40% (Fig. 3B).

3.3. Effect of dopamine on adrenal DOPA and dopamine

The NSD 1015-induced accumulation of DOPA in the adrenals was significantly enhanced, by about 30%, by treatment with the lowest dose of dopamine (1 mg/kg), higher doses of dopamine (3 and 9 mg/kg) being ineffective (Table 1). The effect of 1 mg/kg dopamine was blocked by domperidone (3 mg/kg s.c., Fig. 4).

At the same time the heart dopamine level was very much increased (2000–6000%), indicating uptake of the s.c. administered dopamine. The heart adrenaline levels were somewhat decreased following all three administered doses. The noradrenaline levels in the heart were also reduced following the two higher doses of dopamine (Table 1).

Forebrain dopamine levels were not significantly changed following dopamine administration. NSD 1015

induced, as expected, a decrease in forebrain DOPAC levels.

3.4. Effect of epinine on adrenal dopamine

When administered in a dose of 0.3 mg/kg s.c., the *N*-methyl derivative of dopamine, epinine, induced a significant increase in adrenal dopamine levels. However, higher doses of epinine (1–9 mg/kg) were without effect. The effect of epinine was blocked by domperidone (3 mg/kg s.c., Fig. 5). After the two highest doses of epinine (3 and 9 mg/kg) the heart adrenaline

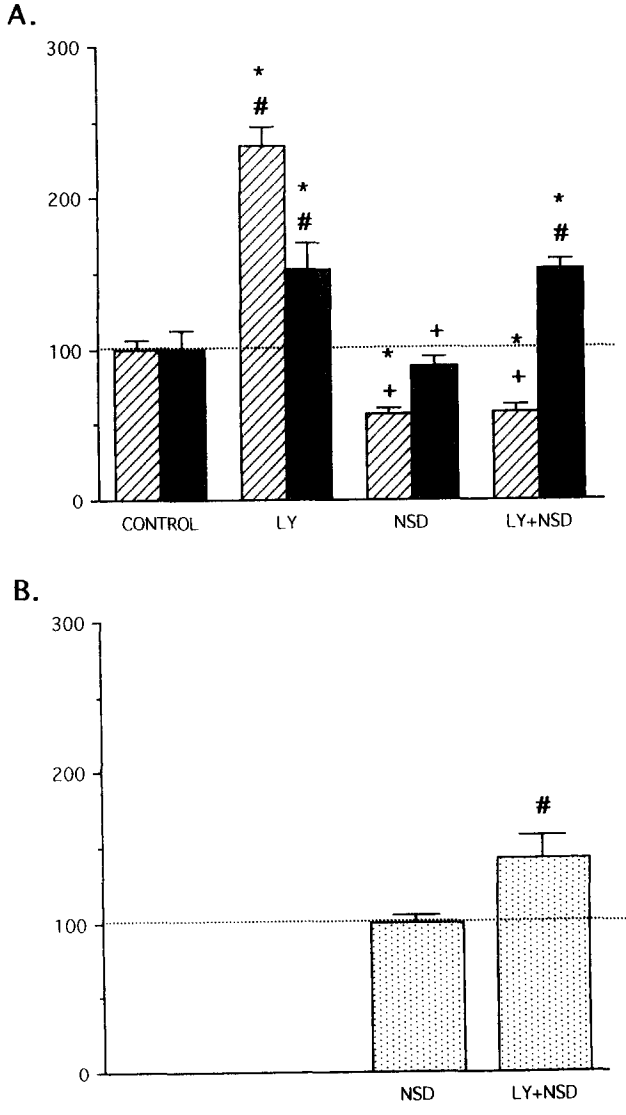

Fig. 3. Aromatic L-amino acid decarboxylase inhibition by NSD 1015 and quinpirole-induced changes in adrenal dopamine and heart adrenaline (A) and adrenal DOPA levels (B) in rats. $n = 5$ in each group. NSD 1015 (NSD), 100 mg/kg s.c. was administered 30 min and quinpirole (LY) 1 mg/kg s.c., 35 min before decapitation. The data are shown as the means ± S.E. and expressed as percentages of the value for the saline-treated controls (A) (138 ± 9 ng/pair adrenal dopamine; 22 ± 3 ng/g heart adrenaline), resp. NSD 1015-treated controls (B) (10 ± 0.4 ng/pair adrenal DOPA). Ordinate: percentage of control levels, $^{*}P < 0.05$ (vs. corresponding control), $^{\#}P < 0.05$ (vs. NSD 1015), $^{+}P < 0.05$ (vs. quinpirole). Hatched columns: adrenal dopamine; filled columns: heart adrenaline; stippled columns: adrenal DOPA.

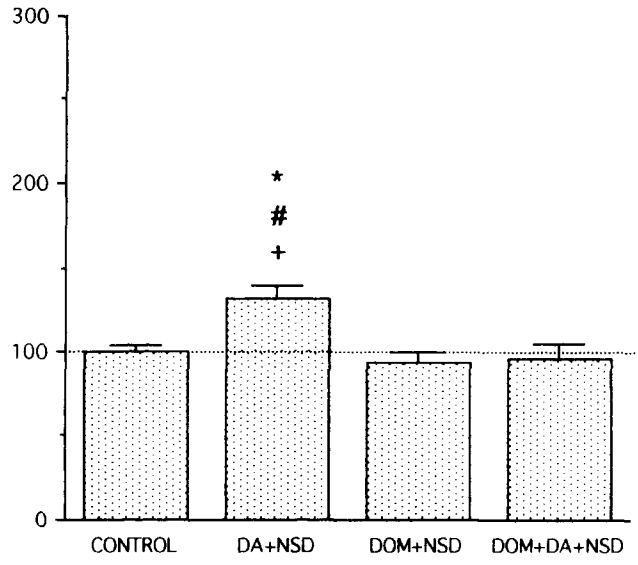

Fig. 4. Effects of domperidone on dopamine-induced changes in adrenal DOPA levels in rats. $n = 5$ in each group. All rats were injected with NSD 1015 (100 mg/kg s.c., 30 min). Domperidone (DOM), 3 mg/kg s.c. was administered 150 min, and dopamine (DA), 1 mg/kg s.c., 35 min before decapitation. The data are shown as the means ± S.E. and expressed as percentages of the value for the NSD 1015-treated controls (12 ± 0.5 ng/pair adrenal DOPA). Ordinate: percentage of control level, $^{*}P < 0.05$ (vs. NSD 1015), $^{\#}P < 0.05$ (vs. domperidone), $^{+}P < 0.05$ (vs. dopamine + domperidone).

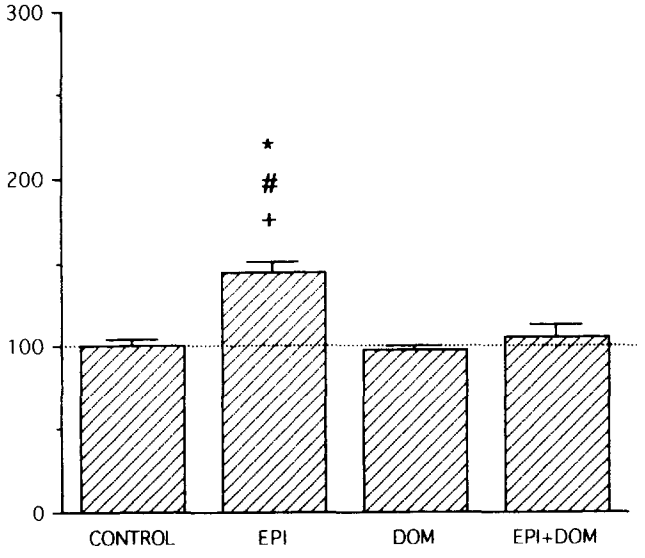

Fig. 5. Effects of domperidone on epinine-induced changes in adrenal dopamine levels in rats. $n = 5$ in each group. Domperidone (DOM), 3 mg/kg s.c. was administered 150 min, and epinine (EPI), 0.3 mg/kg s.c., 30 min before decapitation. The data are shown as the means ± S.E. and expressed as percentages of the value for the saline-treated controls (139 ± 12 ng/pair adrenal dopamine). Ordinate: percentage of control level, $^{*}P < 0.05$ (vs. control), $^{\#}P < 0.05$ (vs. domperidone), $^{+}P < 0.05$ (vs. epinine + domperidone).

Table 1
Dopamine-induced changes in DOPA and dopamine (DA) in the adrenals, noradrenaline (NA), adrenaline (A) and dopamine in the heart and dopamine and DOPAC in the forebrain of rats

	Adrenals		Heart			Forebrain	
	DOPA	DA	NA	A	DA	DA	DOPAC
Control (saline)	–	100 ± 9 (123 ± 11)	100 ± 4 (1030 ± 40)	100 ± 6 (16 ± 1)	100 ± 10 (21 ± 2)	100 ± 8 (1788 ± 143)	100 ± 6 (228 ± 14)
DA-3	–	169 ± 7 [a,b]	84 ± 2	55 ± 3 [a,b]	2524 ± 48 [a,b]	114 ± 13	108 ± 7 [b]
NSD 1015	100 ± 4 (11 ± 0.5)	65 ± 4 [a]	104 ± 6	105 ± 8	60 ± 12	91 ± 8	45 ± 2 [a]
DA-1 + NSD 1015	134 ± 13 [b]	–	103 ± 10	89 ± 18	2027 ± 213 [a,b]	90 ± 4	42 ± 5 [a]
DA-3 + NSD 1015	103 ± 9	146 ± 14 [a,b]	83 ± 4	64 ± 14 [a]	3013 ± 163 [a,b]	91 ± 6	58 ± 5 [a]
DA-9 + NSD 1015	101 ± 7	–	78 ± 6	70 ± 14	6237 ± 1195 [a,b]	87 ± 5	54 ± 7 [a]

$n = 5$ in each group. NSD 1015, 100 mg/kg s.c., was administered 30 min and dopamine (DA), 1, 3 and 9 mg/kg 35 min before decapitation. The data are shown as the means ± S.E. expressed as percentages of the value for the saline-treated controls, with the exception of the adrenal DOPA concentration when NSD 1015 controls were used. Values in parentheses refer to the absolute values for the controls, in ng/g tissue (for adrenals, ng/pair). [a] $P < 0.05$ (vs. corresponding saline-treated control). [b] $P < 0.05$ (vs. NSD 1015).

Table 2
Epinine-induced changes in dopamine (DA) in the adrenals, noradrenaline (NA), adrenaline (A), DOPAC and dopamine in the heart, and dopamine and DOPAC in the forebrain of rats

	Adrenals	Heart				Forebrain	
	DA	NA	A	DOPAC	DA	DA	DOPAC
Control	100 ± 3 (158 ± 5)	100 ± 4 (1030 ± 40)	100 ± 7 (14 ± 1)	100 ± 8 (12 ± 1)	100 ± 7 (15 ± 1)	100 ± 6 (1810 ± 103)	100 ± 4 (271 ± 12)
Epinine – 0.3	152 ± 12 [a]	100 ± 7	108 ± 14	184 ± 21	85 ± 6	97 ± 4	97 ± 6
Epinine – 1	117 ± 6	85 ± 2	125 ± 18	505 ± 49 [a]	102 ± 8	93 ± 9	103 ± 13
Epinine – 3	119 ± 8	71 ± 5 [a]	232 ± 28 [a]	1186 ± 81 [a]	97 ± 13	105 ± 9	110 ± 11
Epinine –9	97 ± 5	44 ± 4 [a]	163 ± 10 [a]	3417 ± 111 [a]	91 ± 15	96 ± 1	102 ± 7 [a]

$n = 4$ in each group. Epinine, 0.3–9 mg/kg s.c., was administered 30 min before decapitation. The data are shown as the means ± S.E. expressed as percentages of the value for the saline-treated controls. Values in parentheses refer to the absolute values for the controls, in ng/g tissue (for adrenals, ng/pair). [a] $P < 0.05$ (vs. corresponding saline-treated control).

levels were significantly increased, whereas the heart noradrenaline levels were significantly decreased. Heart DOPAC levels showed a dose-dependent increase up to 2500%, compared to saline-treated controls (Table 2). No significant changes in either DOPAC or dopamine were observed in the forebrain following epinine treatment (Table 2).

4. Discussion

The present observations support our previous results, indicating that dopamine agonists acting on dopamine D_2-like receptors are capable of stimulating adrenal catecholamine synthesis and that the receptors involved are located peripherally. Furthermore, they confirm our previous assumption that the effect is mediated via stimulation of tyrosine hydroxylase, i.e. the first, rate-limiting enzyme catalysing the synthesis of catecholamines. In addition the present study provides evidence that dopamine itself is capable of stimulating the first step of adrenal catecholamine synthesis. This appears to be true also of epinine, i.e. the *N*-methyl derivative of dopamine, which like dopamine is known to be virtually unable to penetrate through the blood-brain barrier.

The elevation of adrenal dopamine produced by epinine is most likely due to an increase in the synthesis of dopamine by the normal metabolic pathway, rather than to *N*-demethylation of epinine, because the latter process does not seem to take place to an appreciable extent (Pocchiari et al., 1986). In fact, this is supported by the present data showing an absence of dopamine elevation in the heart following epinine treatment. The elevation of DOPAC and adrenaline in the heart following epinine treatment indicates that there are two major metabolic pathways for epinine, i.e. oxidative deamination and beta-hydroxylation (Bridgers and Kaufman, 1962).

In the present study the poor penetration of dopamine into the brain was strikingly demonstrated by the lack of significant changes in forebrain dopamine levels, while at the same time the dopamine levels in the heart rose dramatically following administration of dopamine. The much lower elevation of dopamine in the adrenals than in the heart may be accounted for by the presence of an active uptake mechanism for amines in adrenergic nerves; in the adrenal medulla such a mechanism, if it exists, appears to be much less powerful.

Similarly the absence of an elevation of DOPAC levels in the forebrain after administration of epinine, in contrast to the dramatic increase in the heart, confirms the poor penetration of this catecholamine into the brain.

The reason why the stimulatory influence of dopamine and epinine on catecholamine synthesis showed up only after the lowest dose may be that higher doses cause a sufficient accumulation of free catecholamines in the adrenomedullary cells to induce the well-known inhibitory action of catechols on tyrosine hydroxylase (Nagatsu et al., 1964).

In conclusion, the present data indicate that stimulation of peripherally located dopamine D_2-like receptors can enhance the rate of adrenal catecholamine synthesis by stimulating the activity of tyrosine hydroxylase.

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