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FORMATION OF DOPAMINE AND NORADRENALINE IN RAT VAS DEFERENS: COMPARISON WITH GUINEA-PIG VAS DEFERENS

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1 The formation of [^{14}C]-3,4-dihydroxyphenylalanine (DOPA) from [^{14}C]-tyrosine, in the presence of the amino acid decarboxylase inhibitor, brocresine (3-hydroxy-4-bromobenzyloxy-amine dihydrogen phosphate), was greatly enhanced in rat vasa deferentia depolarized by a KCl-enriched Krebs-Henseleit solution (52 mM KCl) compared with tissues maintained in unmodified Krebs-Henseleit solution.

2 When the conversion of tyrosine was allowed to proceed as far as catecholamine (brocresine absent) no significant difference was observed between the accumulation of [^{14}C]-catecholamines (CA) in depolarized rat vasa deferentia and the accumulation in control (non-depolarized) tissues.

3 Endogenous CA levels in the depolarized rat vasa deferentia fell to 67% of the controls after a 1 h incubation period and to 53% at the end of 2 hours.

4 Chromatographic separation on Amberlite CG-120 columns of the newly synthesized CA and catechol metabolites from the rat vas deferens revealed that a very high proportion was present as dopamine. The percentage distribution after 1 h incubation in control Krebs-Henseleit was: noradrenaline (NA): 30.6 ± 5.2; dopamine 56.9 ± 5.9; acid metabolites: 12.8 ± 1.1; and in KCl-rich Krebs-Henseleit, NA: 32; dopamine: 44.7 and acid metabolites 23.3. In contrast to the newly synthesized (^{14}C-labelled) CA, endogenous dopamine comprises only 10% of the endogenous CA stores in rat vas deferens.

5 The distribution of newly synthesized NA and dopamine in rat vas deferens is strikingly different from that of guinea-pig vas deferens where more than 80% of newly formed amine is present as NA. In the latter tissue depolarization with K^+ causes a striking increase in CA biosynthesis.

Introduction

The ability of many sympathetically-innervated tissues to maintain endogenous stores of noradrenaline (NA) under various conditions of physiological activity arises from the combined effects of the increased formation of NA that takes place during nerve stimulation (see Weiner, 1970) and the recapture of released transmitter at the nerve ending (Iversen, 1967). If either of these processes is inhibited a rapid fall in tissue content of NA may occur. Some sympathetically-innervated tissues can, however, be partially depleted of their NA stores when subjected to prolonged nerve stimulation alone. For example, Dearnaley & Geffen (1966) were able to produce a reduction in the NA content of the spleen of the cat by stimulating the splenic nerve electrically and Chang & Chang (1965) also obtained a fall in endogenous NA in rat vas deferens by stimulation of postganglionic fibres. More recently Bisby & Fillenz (1969) reported that perfusion of the rat vas deferens with a Krebs medium containing 127 mM K resulted in a 20% decrease in the NA content of the rat vas deferens. The depletion of NA is usually only seen with prolonged stimulation at relatively high frequencies. Part of the explanation for this may lie in the fact that although the efficiency of the reuptake process is maintained over a wide range of frequencies of stimulation (Hughes, 1972) there is nonetheless a larger amount of NA released, and therefore also lost, at the higher frequencies. It has been proposed that more fibre terminals are activated at high frequencies of stimulation (Hughes, 1972). In the rat vas deferens depolarized with a K-rich medium there may also be another factor involved. In the present study we have found that there is no net increase in the accumulation of newly formed catecholamine (CA) in depolarized vasa

deferentia when compared with controls even though there is an increase in the formation of [^{14}C]-DOPA from [^{14}C]-tyrosine. This is in marked contrast to the guinea-pig vas deferens where depolarization by K increases the amount of newly formed CA in the tissue by 100%. Another unusual finding in the rat vas deferens is the large proportion of newly formed CA present as dopamine rather than as NA.

Methods

Vasa deferentia dissected from rats killed by a blow on the head were incubated with [^{14}C]-tyrosine (final concentration 5×10^{-5} M; specific activity 10 mCi/mM) in Krebs-Henseleit bicarbonate medium bubbled with 95% O_2 and 5% CO_2 and maintained at 37°C or in a Krebs-Henseleit medium in which various proportions of NaCl were replaced by equimolar amounts of KCl. For most of the experiments 40% of the NaCl was replaced by KCl in the KCl-enriched medium giving final concentrations of NaCl and KCl of 70.84 mM and 52 mM respectively. The unmodified medium had the following composition (mM): NaCl 118.07, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25.00, glucose 11.01, disodium edetate (EDTA) 0.027. At the end of the incubation the tissues were blotted rapidly, frozen on solid CO_2, weighed and homogenized in ice cold 15% trichloroacetic acid (1 to 3, w/v). The homogenate was centrifuged at 10,000 rev/min for 10 min (4°C) (Sorvall RC2B refrigerated centrifuge) and the catecholamines and catechol metabolites isolated from the supernatants by alumina chromatography (Boadle-Biber, Hughes & Roth, 1970). The incubation media were also analysed for [^{14}C]-CA and labelled deaminated metabolites by a slight modification of this procedure (Boadle-Biber & Roth, 1972a). Aliquots of the alumina eluates were used to determine endogenous NA with a modification of the fluorimetric method of von Euler & Lishajko (1961) (Boadle-Biber *et al.*, 1970), dopamine by the procedure of Laverty & Taylor (1968) with minor changes (Boadle-Biber & Roth, 1972b) and total catechol radioactivity by liquid scintillation spectrometry (see Boadle-Biber *et al.*, 1970 for details). Values for CA formation which are expressed as d min^{-1} g^{-1} fresh tissue ± s.e. mean were corrected for a recovery from the alumina of 82%. The corrections applied for endogenous NA and dopamine were 83% and 81% respectively.

In order to determine the contribution of NA and dopamine to the newly formed catechol compounds the amines isolated by alumina chromatography from two rat vasa deferentia were separated by ion exchange chromatography on Amberlite CG-120 columns (Stjärne & Lishajko, 1967). To obtain a sufficiently high level of radioactivity for the ion exchange chromatography the tissues were incubated for 1 h with [^{14}C]-tyrosine having a specific activity of 50 mCi/mM. The final concentration (5×10^{-5} M) was the same as in earlier experiments. To identify the position of the ^{14}C-catechol compounds coming off the resin column cold carriers (DOPA, NA, dopamine) were added to the PCA eluate prior to chromatography on the Amberlite resin, and their native fluorescence at 335 nm (excitation wavelength 285 nm) determined using an Aminco Bowman spectrophotofluorimeter. Radioactivity was determined by counting 1.0 ml aliquots of the pooled fractions of the acid eluate in 10 ml of a scintillation fluid (DTE, see Boadle-Biber *et al.*, 1970) using a Packard scintillation counter.

In a further experiment a comparison was made between the elution profiles of newly synthesized NA and dopamine isolated from guinea-pig and rat vas deferens. The tissues were incubated with [^{14}C]-tyrosine for 1 h in control Krebs-Henseleit solution. In the case of the guinea-pig vas deferens a specific activity of 50 mCi/mM and final concentration of tyrosine of 5×10^{-5} M was used. However, with the rat vas deferens, in order to obtain a sufficient amount of radiolabelled CA to follow the elution profiles for [^{14}C]-NA and [^{14}C]-dopamine undiluted [^{14}C]-tyrosine, 300 mCi/mM, 5 μCi/ml incubation medium and final concentration of 1.67×10^{-6} M, had to be used.

The elution profiles for the two radiolabelled amines were followed by counting 1.0 ml aliquots from each fraction (1.5 ml) of acid eluate obtained from the Amberlite column in 10 ml scintillation fluid as described earlier (Boadle-Biber *et al.*, 1970).

In one experiment the conversion of [^{14}C]-tyrosine to [^{14}C]-DOPA was followed by incubating the tissue in the presence of the aromatic amino acid decarboxylase inhibitor, brocresine, in a final concentration of 10^{-4} M. At this concentration more than 95% of the ^{14}C-catechol compounds isolated by alumina chromatography and identified by chromatography on Amberlite CG-120 was present as [^{14}C]-DOPA. The recovery of DOPA eluted from the alumina columns with 4.0 ml 0.5 N HCl, was 88%.

The specific activity of tyrosine present in the tissue at the end of the incubation was determined

in some experiments using methods described earlier (Boadle-Biber *et al.*, 1970; Boadle-Biber & Roth, 1972a).

Results

The net accumulation of ^{14}C-CA from tyrosine in the rat vas deferens proceeded in a linear manner for up to 1 h in both control and 52 mM KCl Krebs-Henseleit media (Table 1a). Thereafter the net accumulation fell off in both media, but the fall was most pronounced in the K-enriched medium. No significant difference was observed in the amount of ^{14}C-CA formed and retained in control tissues and those incubated in K-rich media during the first hour of incubation (Table 1b). Increasing the proportion of NaCl replaced by KCl resulted in a drop in the content of ^{14}C-CA isolated from the tissues incubated in the K-rich media to values below that for the control, an effect which may have arisen in part from the decrease in the specific activity of the tyrosine present in these tissues (Table 2). There was an overall loss of endogenous NA with time in tissues incubated in 52 mM KCl Krebs-Henseleit medium (Table 1b) and this loss of NA was further enhanced when the proportion of KCl in the medium was increased (Table 2). No intact endogenous NA could be detected in the bath fluid, however, by the fluorimetric assay. In contrast to the finding on ^{14}C-CA synthesis, formation of [^{14}C]-DOPA was enhanced in the 52 mM KCl medium (Table 1a). This observation prompted us to identify the newly formed CA present in the tissue by Amberlite CG-120 ion exchange chromatography: CA comprised over 70% of the total labelled catechols and of this, more than half was present as dopamine (Table 3). The elution profile of the labelled CA was checked against the peak of the native fluorescence of the added carriers NA and dopamine. Its distribution was also compared with that for newly formed CA isolated from guinea-pig vas deferens; in the case of the guinea-pig 88% of the labelled catechol material was NA and only 3.5% dopamine (Figure 1). Determinations of endogenous dopamine levels in the rat vas deferens revealed that this amine comprised approximately 10% of the total tissue CA.

Table 1a Synthesis of ^{14}C-catecholamines and [^{14}]-DOPA from [^{14}C]-tyrosine in rat vasa deferentia incubated in control or 52 mM KCl Krebs-Henseleit solution

Time		*Net accumulation of ^{14}C-CA from [^{14}C]-tyrosine (d min^{-1} g^{-1} fresh tissue ± s.e. mean)*			*Net accumulation of [^{14}C]-DOPA^{+} from [^{14}C]-tyrosine (d min^{-1} g^{-1} fresh tissue ± s.e. mean)*			
(h)	n*	*Control Krebs*	n	*52 mM KCl Krebs*	n**	*Control Krebs*	n	*52 mM KCl Krebs*
0.5	6	7514 ± 1012	6	7954 ± 673	3	7297 ± 1292	3	28090 ± 4674
1.0	21	15110 ± 1067	18	16937 ± 1120	3	14235 ± 1650	3	34391 ± 4465
1.5	3	18876 ± 3185	3	14126 ± 1194				
2.0	6	21199 ± 3176	6	11536 ± 673				

Table 1b Endogenous catecholamines (CA) remaining in rat vasa deferentia after different incubation times in control or 52 mM KCl Krebs-Henseleit solution

	Endogenous CA (μg/g fresh tissue ± s.e. mean)							
	Noradrenaline				*Dopamine*			
Time (h)	n	*Control Krebs*	n	*52 mM KCl Krebs*	n	*Control Krebs*	n	*52 mM KCl Krebs*
0.5	6	9.88 ± 0.72	6	8.30 ± 0.94	–	–	–	–
1.0	22	7.81 ± 0.62	22	5.25 ± 0.40	4	1.00 ± 0.09	4	0.54 ± 0.04
2.0	6	7.30 ± 0.69	6	3.86 ± 0.42	–	–	–	–

*n = number of individual vasa deferentia analysed for ^{14}C-CA, catechol metabolites and endogenous NA and dopamine.

**n = number of individual vasa deferentia and bath fluids analysed for [^{14}C]-DOPA.

$^{+}$ Vasa deferentia were incubated in the presence of the aromatic amino acid decarboxylase inhibitor, brocresine, (10^{-4} M). More than 95% of labelled catechols were found to be present as [^{14}C]-DOPA when analysed by Amberlite CG-120 chromatography.

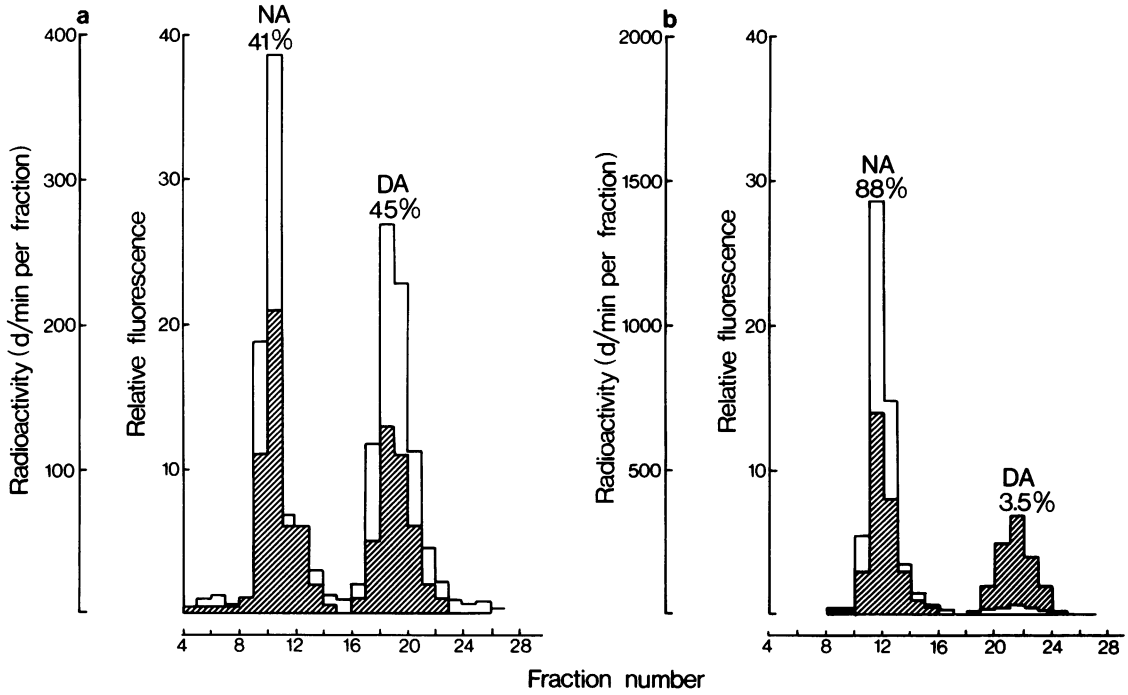

Figure 1 ^{14}C-catecholamines isolated from single pairs of (a) rat and (b) guinea-pig vasa deferentia by alumina chromatography were separated by ion exchange chromatography using Amberlite CG-120 resin according to the method of Stjärne & Lishajko (1967). Cold carriers dopamine (DA) and noradrenaline (NA) were used to identify the amine peaks by native fluorescence at 335 nm (excitation 285 nm). Open columns, radioactivity; cross-hatched columns, relative fluorescence.

Discussion

The observation that there is no net increase in the content of ^{14}C-CA or [^{14}C]-NA in depolarized rat vasa deferentia compared with controls, even though the formation of [^{14}C]-DOPA is enhanced, may be accounted for in part by preferential and rapid release of newly formed (i.e. radiolabelled) NA and in part by the accumulation of dopamine within the cytoplasm and its subsequent

Table 2 Effect of increasing concentrations of KCl on the formation of ^{14}C-catecholamines (CA) from [^{14}C]-tyrosine in the rat vas deferens incubated for 1 hour.

% NaCl replaced with KCl	n*	*Net accumulation of ^{14}C-CA (d/min^{-1} g^{-1} tissue in 1 h ± s.e. mean)*	n	*Endogenous NA (μg/g fresh tissue ± s.e. mean)*	n	*Specific activity of tyrosine (d/min [^{14}C]-tyrosine/μg tyrosine ± s.e. mean)*	*Release of ^{14}C-catechol into bath⁺ (% total d/min in control)*
0	21	15110 ± 1067	22	7.81 ± 0.62	4	34932 ± 2550	100 (3627)
20	4	16431 ± 1519	4	5.07 ± 0.68	4	38601 ± 2978	120
40	18	14126 ± 1194	22	5.25 ± 0.40	4	31006 ± 1853	246
60	4	11707 ± 1617	4	3.08 ± 0.17	4	25444 ± 777	214
80	4	9305 ± 938	4	3.33 ± 0.39	4	26788 ± 1894	254

⁺ Release of ^{14}C-catechols is expressed as % of the total catechol d/min present in the control media. No release of endogenous NA could be detected by fluorimetric assay.

*n = Number of individual vasa deferentia analysed.

deamination by monoamine oxidase and loss from the tissue. Analysis of the incubation media revealed that there is an increase in ^{14}C-catechol compounds present in K-rich media. However, they have not been identified and so it is not known whether they are present as intact dopamine, NA or acidic or neutral metabolites of these amines. The failure of the tissue to form or retain increased amounts of CA during depolarization clearly contributes to the net fall in endogenous amine levels seen in the vas deferens of the rat under these conditions (Table 1b; cf. Bisby & Fillenz, 1969).

The finding that such a high proportion of newly formed CA in rat vas deferens is present as [^{14}C]-dopamine is quite unexpected and is in contrast to the situation found in other sympathetically-innervated tissues where [^{14}C]-NA is the predominant CA formed from [^{14}C]-tyrosine (e.g. Alousi & Weiner, 1966; Roth, Stjärne & von Euler, 1966, 1967; Boadle-Biber *et al.*, 1970). A comparison of the proportion of [^{14}C]-dopamine found in guinea-pig vas deferens incubated in control Krebs-Henseleit medium under identical experimental conditions revealed that only 3.5% of the total catechol compounds was present as [^{14}C]-dopamine whereas 88% was in the form of NA (Figure 1). In rat vas deferens [^{14}C]-dopamine represented between 45 and 60% of the total labelled catechols. This large accumulation of [^{14}C]-dopamine which makes up more than half of the newly formed CA does not, however, reflect the contribution of dopamine to the endogenous stores of CA. Endogenous dopamine makes up only 10% of the total CA; the other 90% is present as NA

The reason for this accumulation of [^{14}C]-dopamine is not clear but a number of possibilities such as saturation of the uptake mechanism for dopamine into the storage vesicle or saturation of dopamine-β-hydroxylase, the enzyme present in the storage vesicle (Hörtnagl, Hörtnagl & Winkler, 1969; DePotter, Smith & DeSchaepdryver, 1970) that converts dopamine to NA, seem likely. Loss of dopamine-β-hydroxylase from the vesicle during the release process (Gewirtz & Kopin, 1970; Smith, DePotter, Moerman & DeSchaepdryver, 1970; Weinshilboum, Thoa, Johnson, Kopin & Axelrod, 1971) could account for a reduction in the conversion of dopamine to NA in depolarized tissues, but does not explain the accumulation of [^{14}C]-dopamine which also takes place in rat vas deferens incubated in control Krebs-Henseleit solution.

Some caution should obviously be exercised when looking at the formation of radiolabelled CA from the amino acid precursor, tyrosine, in sympathetically-innervated tissues; any assumptions about the identity of the newly formed CA based on results from other sympathetically-innervated tissues may prove misleading.

Finally, these results may explain why it is difficult to demonstrate a depolarization-induced increase in CA biosynthesis in the rat vas deferens in contrast to the pronounced and highly reproducible acceleration of CA biosynthesis which is readily observed on depolarization of the guinea-pig vas deferens (Boadle-Biber *et al.*, 1970).

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Table 3 Analysis* by Amberlite CG-120 chromatography of ^{14}C-catechol compounds formed from [^{14}C]-tyrosine in rat vasa deferentia incubated in control or 52 mM KCl Krebs-Henseleit solution.

	Control Krebs-Henseleit			*52 mM KCl Krebs-Henseleit*		
Time	*Acid metabolites*	*NA*	*Dopamine*	*Acid metabolites*	*NA*	*Dopamine*
(h)	*%*	*%*	*%*	*%*	*%*	*%*
0.5	25.5	20.1	54.4	23.8	26.0	50.1
	17.0	22.2	60.7	20.6	23.3	56.1
1.0	11.8	25.6	62.5	23.0	34.2	42.9
	11.6	25.2	63.1	23.6	29.9	46.5
	15.0	41.0	45.0			
	12.8 ± 1.1	30.6 ± 5.2	56.9 ± 5.9			

* ^{14}C-catechol compounds were isolated by alumina chromatography and then identified by Amberlite CG-120 chromatography.

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