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# CATECHOLAMINE SYNTHESIS IN RABBIT CAROTID BODY *IN VITRO*

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## SUMMARY

1. Catecholamine synthesis in rabbit carotid body was studied *in vitro* using [$^3$H]DOPA and [$^3$H]tyrosine as precursors. The effects of sympathectomy and transection of the carotid sinus nerve on [$^3$H]dopamine ([$^3$H]DA) and [$^3$H]noradrenaline ([$^3$H]NA) synthesis were investigated in chronically denervated carotid bodies.

2. When [$^3$H]DOPA was used as precursor, the synthesis of [$^3$H]DA was linear for more than 6 hr. The carotid body synthesized larger amounts of [$^3$H]catecholamines than when [$^3$H]tyrosine was used as precursor, but most of this excess was liberated into the incubation media. Using 10 $\mu$M-[$^3$H]DOPA as precursor, the synthesis rates were 6·76 and 1·51 n-mole/g per hr for [$^3$H]DA and [$^3$H]NA, respectively; with 40 $\mu$M-[$^3$H]DOPA, these values increased to 19·22 and 3·23 n-mole/g per hr, respectively.

3. The relationship between [$^3$H]DOPA concentration and [$^3$H]DA synthesis was linear throughout the range 5–40 $\mu$M-[$^3$H]DOPA.

4. Sympathectomy reduced the synthesis of [$^3$H]NA by 90 % and [$^3$H]DA by 37 % when [$^3$H]DOPA was used as precursor.

5. When [$^3$H]tyrosine (40 $\mu$M) was used as precursor, synthesis of [$^3$H]catecholamines was linear for at least 4 hr, with rates of 12·10 and 0·85 n-mole/g per hr for [$^3$H]DA and [$^3$H]NA, respectively.

6. [$^3$H]DA and [$^3$H]NA synthesis from [$^3$H]tyrosine exhibited the characteristics of saturable processes, with $K_m$ values of 16·8 and 17·6 $\mu$M, respectively.

7. 6-methyltetrahydropterine (6-MPH$_4$, 100 $\mu$M), a synthetic analogue of the natural co-factor for tyrosine hydroxylase, increased [$^3$H]DA and [$^3$H]NA synthesis from [$^3$ H]tyrosine in both the carotid body and superior cervical ganglion, with the greatest effect seen in the carotid body.

8. When [$^3$H]tyrosine was used as precursor, sympathectomy of the carotid body reduced [$^3$H]NA synthesis by 80 %, but did not alter [$^3$H]DA or [$^3$H]tyrosine levels in the tissue. Transection of the carotid sinus nerve had no effect on [$^3$H]catecholamine synthesis in the carotid body.

## INTRODUCTION

The carotid body is a catecholamine-rich organ containing primarily dopamine (DA) and noradrenaline (NA; Chiocchio, Biscardi & Tramezzani, 1966; Dearnaley, Fillenz & Woods, 1968; Zapata, Hess, Bliss & Eyzaguirre, 1969; Hellstrom & Koslow,

1975). On the basis of ultrastructural (Lever & Boyd, 1957) and histofluorescence (Chiocchio, King & Angelakos, 1971) studies, the type I cells have been identified as the principal site of catecholamine storage in this organ. Although there is now increasing pharmacological and biochemical evidence implicating DA in the response of carotid chemoreceptors (Black, Comroe & Jacobs, 1972; Sampson, Aminoff, Jaffe & Vidruk, 1976; Zapata, 1975; Hanbauer & Hellstrom, 1978), most probably as a neurotransmitter or 'modulator' of afferent-fibre discharge (Fillenz, 1975; Llados & Zapata, 1978*a*, *b*), the synthesis, storage and release of DA as a function of chemoreceptor activity have not yet been characterized systematically.

In previous studies on rabbit carotid body, we measured the activity of the rate-limiting enzyme of catecholamine synthesis, tyrosine hydroxylase, and showed further that hypoxia resulted in a long-term increase in the activity of this enzyme (Gonzalez, Kwok, Gibb & Fidone, 1981). In this paper and the following two reports we extend our studies on the role of catecholamines in rabbit carotid body to a consideration of their metabolism in relation to alterations in chemosensory activity. The present paper deals with the kinetics of DA and NA synthesis from their precursors, tyrosine and DOPA, and the effects of transection of the carotid nerve and sympathectomy on the synthesis.

## METHODS

*Tissue incubation and sample preparation.* Rabbits (New Zealand White, 2–3 kg body wt.) were housed in a controlled temperature (22 °C) vivarium with normal dark/light rhythm and *ad libitum* feeding. Animals were anaesthetized with sodium pentobarbitone (30 mg/kg, Diabutal), administered I.P., and supplemented as necessary through the lateral ear vein. The trachea was cannulated, the region of the carotid bifurcation surgically exposed, and the carotid body removed and placed in a dissecting chamber filled with ice-cold physiological saline. The carotid body was quickly cleaned of surrounding connective tissue and immediately transferred to incubation vials in a metabolic shaker (New Brunswick Scientific) at 37 °C for periods ranging from 30 min to 6 hr; each vial contained 2 ml. modified Tyrode solution (mM): NaCl, 112; KCl, 4·7; $CaCl_2$, 2·2; $MgCl_2$, 1·1; sodium glutamate, 42; HEPES, 5; ascorbic acid, 1; glucose, 5·6; Baron & Eyzaguirre, 1977) at pH 7·4, equilibrated with 100 % $O_2$ prior to addition of ascorbic acid. In addition, the incubation media contained a labelled precursor for catecholamine synthesis, either [$^3$H]tyrosine (10, 20, 40 or 60 μM-L-[3,5-$^3$H]tyrosine, 2 Ci/mM, Amersham) or [$^3$H]DOPA (5, 10, 20 or 40 μM-L-3,4-dihydroxy [ring −2,5,6-$^3$H]phenylalanine, 1 Ci/mM, Amersham). Following incubation, the tissues were placed for 3 min in vials filled with ice-cold Tyrode solution (equilibrated with 100 % $O_2$) to allow for wash-out of extracellular label (half-time of [$^3$H]inulin wash-out from the carotid body: approximately 1 min). Each carotid body was weighed on a Cahn electrobalance (sensitivity, ±50 ng) after lightly blotting the tissue to remove excess Tyrode solution. The weighing chamber was provided with a humidified atmosphere (100 % relative humidity) to prevent drying of the tissue during the weighing procedure (mean weight of rabbit carotid bodies = 388 ± 129 (S.D.) μg). Tissue samples were placed on small flat mortars (1 cm ground-glass plates) and frozen rapidly to −12 °C in a drop (20 μl.) of extraction/carrier mixture containing 0·3 N-acetic acid, 20 mM-ascorbic acid, and 4 mM-unlabelled DA and NA in Tyrode solution (pH 3·6, modified from Besson, Cheramy, Feltz & Glowinski, 1969). This medium gave slightly higher and more consistent catecholamine yields than a 0·4 N-perchloric acid/carrier mixture (Nagatsu, 1973), and provided excellent stability of the catecholamines during storage, even for long periods of time (1–3 months).

*Separation of catecholamines.* High-voltage paper electrophoresis was used to separate the catecholamines and their precursors (Nagatsu, Levitt & Udenfriend, 1964; Hildebrand, Barker, Herbert & Kravitz, 1971). Prior to electrophoresis (32 V/cm for 5 hr 40 min on Whatman no. 1 paper at 2–4 °C), the tissue samples were thawed and homogenized on their mortar plates using small ground-glass pestles; the plates were then inverted onto separate channels at the electrophoretic

origin. A high ionic strength buffer (acetic acid, 0·23 M; formic acid, 0·25 M; ammonium hydroxide, 0·25 M; pH 4·5) permitted good separation with very little tailing; contamination in a single channel between DA and NA was less than 5%, and spill-over between 2 in-wide channels was negligible (i.e. when [$^3$H]DA was electrophoresed to give approximately 25,000 counts/min in the DA peak, adjacent blank channels had background level counts in the corresponding DA areas). Recovery of preformed [$^3$H]DA and [$^3$H]NA after electrophoresis from unincubated tissue homogenates was approximately 95%.

Following electrophoresis, the channels were led through a radiochromatogram scanner (Packard 7201) and the peaks for DA, NA, tyrosine and DOPA were identified by comparison of relative electrophoretic mobilities with standards run in separate channels. The regions of the electrophoretic channels on the experimental radiochromatograms corresponding to the given peaks on the standard radiochromatograms were cut out and combusted in a sample oxidizer (Packard 306; tritium recovery, > 99%) to yield tritiated water in an aqueous counting cocktail (Oxifluor $H_2O$, New England Nuclear). The samples were counted in a liquid scintillation spectrometer (Packard 3385) and d/min were calculated from quench correction curves constructed using [$^3$H]hexadecane standards through the sample combustion and counting steps. Catecholamine and precursor concentrations are expressed in n-mole/g tissue (±S.E.), and the data were evaluated using Student's *t* test.

Recovery of catecholamines from the incubation media was accomplished using a standard alumina adsorption method (Crout, 1961), followed by electrophoretic separation, sample combustion and counting as described above for the tissue samples. In separate experiments designed to test for non-enzymatic formation of labelled catecholamines from their labelled precursors, $10^{-4}$ M-α-methyl-*p*-tyrosine (Sigma; DL-methyl ester HCl) was added to the incubation media along with 60 μM-[$^3$H]tyrosine; this completely inhibited the synthesis of [$^3$H]DA and [$^3$H]NA, and therefore non-enzymatic formation of catecholamines was unlikely in these experiments.

*Denervations.* In some experiments, the superior cervical ganglion or a 3–5 mm length of carotid sinus nerve was excized unilaterally from the rabbit 12–15 days prior to experimentation. Sham-operated carotid bodies on the contralateral side in these animals served as control. Denervations were performed under aseptic surgery, and the rabbits received 0·5 ml. penicillin G procaine (Duracillin, Lilly) I.M. and 10 ml. glucose–saline (5% dextrose in 0·5% NaCl, McGaw) S.C.

## RESULTS

### [$^3$*H*]*catecholamine formation from* [$^3$*H*]*DOPA*

*Time course of synthesis.* When carotid bodies were incubated with 10 μM-[$^3$H]DOPA, the accumulation of [3H]DA in the tissue was linear with time during a 6 hr period of incubation (Fig. 1*A*, filled triangles; 6·76 n-mole/g per hr, $r = 0.998$). [$^3$H]NA accumulated in the tissue at slower rates than [$^3$H]DA (Fig. 1*A*, filled circles), reflecting a slower turnover rate and smaller pool size of NA than DA in the organ. This concentration of DOPA was chosen for these experiments because studies in other catecholaminergic systems have shown that synthesis is negligible with DOPA concentrations much below $10^{-5}$ M (Levitt, Spector, Sjoerdsma & Udenfriend, 1965), a finding consistent with the high $K_m$ of L-aromatic amino acid decarboxylase. The turnover times of [$^3$H]DA and [$^3$H]NA synthesized from 10 μM-[$^3$H]DOPA in rabbit carotid body can be determined from the data in Fig. 1*A* and the values for endogenous DA and NA concentrations in this tissue (DA, 30 μg/g; NA, 1·5 μg/g; Dearnaley *et al.* 1968); the turnover times thus calculated are 29·0 hr for DA and 5·9 hr for NA, the shorter turnover time for NA reflecting the smaller pool size and the preferential use of DOPA as precurser in NA-containing structures (Udenfriend, Zaltzman-Nirenberg, Gordon & Spector, 1966; Persson, 1969).

When [$^3$H]DOPA was used as precursor, large amounts of [$^3$H]DA and [$^3$H]NA could be recovered from the incubation media; e.g. after 3 hr in 10 μM-[$^3$H]DOPA,

305·9 ± 14·8 n-mole [$^3$H]DA/g tissue and 76·2 ± 10·7 n-mole [$^3$H]NA/g tissue were recovered from the media. These data agree with studies by Weiner & Rabadjija (1968), who showed that when the rate-limiting step for catecholamine synthesis in guinea-pig vas deferens was obviated by use of DOPA in place of tyrosine, much of the newly synthesized catecholamines was liberated into the medium.

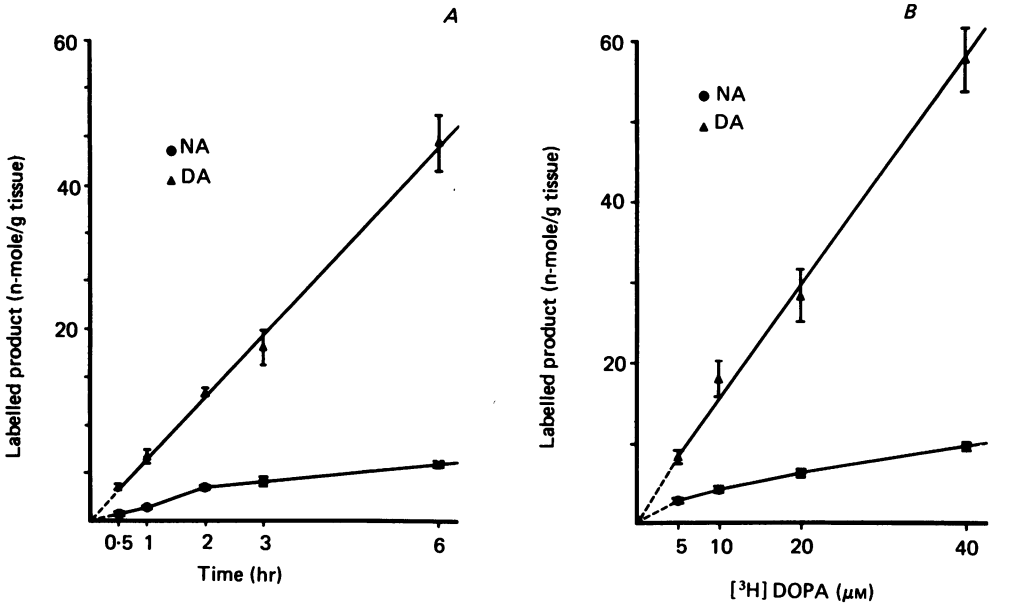


Fig. 1. *A*, time course of synthesis of [$^3$H]DA and [$^3$H]NA in rabbit carotid body using 10 μM-[$^3$H]DOPA as precursor. *B*, relationship between [$^3$H]DOPA concentration and synthesis of [$^3$H]DA and [$^3$H]NA in rabbit carotid bodies incubated for 3 hr with precursor compound.

Carotid bodies were incubated for 3 hr with concentrations of [$^3$H]DOPA 5–40 μM. As shown in Fig. 1*B*, synthesis of [$^3$H]DA and [$^3$H]NA was approximately linear throughout this concentration range, an observation similar to that found for sympathetic endings of guinea-pig heart (Levitt *et al.* 1965). These data suggest a high $K_m$ for DOPA decarboxylase in the carotid body, in agreement with that described for this enzyme in other catecholaminergic structures (Levitt *et al.* 1965).

*Effects of sympathectomy.* Sympathectomized carotid bodies were excized along with their contralateral normally innervated controls and incubated for 2 hr with 10 μM-[$^3$H]DOPA. Comparison of the [$^3$H]NA and [$^3$H]DA content in sympathectomized *vs.* control carotid bodies revealed a 90% reduction in [$^3$H]NA (3·59 ± 0·23 *vs.* 0·37 ± 0·07 n-mole/g tissue; $P < 0·001$) and a 37% reduction in [$^3$H]DA (13·39 ± 0·25 *vs.* 8·50 ± 0·37 n-mole/g tissue; $P < 0·01$) in sympathectomized organs. The decrease of [$^3$H]DA in sympathectomized organs was not surprising, since it has been shown that sympathetic nerve terminals accumulate significant amounts of DA when DOPA is used as precursor (Levitt *et al.* 1965; Persson, 1969; Jonsson & Sachs, 1970).

*[$^3$H]catecholamine formation from [$^3$H]tyrosine*

*Effects of co-factor on the synthesis of catecholamines.* The availability of the co-factor for tyrosine hydroxylase, tetrahydrobiopterin, has been shown to be a limiting factor for catecholamine synthesis in brain (Kettler, Bartholini & Pletscher, 1974). It was thus of interest in our experiments to determine whether exogenous co-factor would modify the synthesis of catecholamines in the carotid body. In these experiments,

TABLE 1. Effects of 6-MPH$_4$ on [$^3$H]catecholamine synthesis from [$^3$H]tyrosine in rabbit carotid bodies and superior cervical ganglia

| | $^3$H-DA | | $^3$H-NA | |
|---|---|---|---|---|
| Tissue | Control | 6-MPH$_4$ | Control | 6-MPH$_4$ |
| Carotid body | 8·06 ± 1·1 | 14·87 ± 1·38* | 0·15 ± 0·02 | 0·68 ± 0·10* |
| Superior cervical ganglion | 2·25 ± 0·07 | 2·95 ± 0·23† | 6·23 ± 0·86 | 7·92 ± 0·90† |

Values are expressed as n-mole/g tissue (± S.E.). Concentration of 6-MPH$_4$, 100 $\mu$M; incubation time, 2 hr in 10 $\mu$M-[$^3$H]tyrosine.

* $P < 0·01$; † $P < 0·05$.

carotid bodies were incubated for 2 hr in Tyrode media containing 10 $\mu$M-[$^3$H]tyrosine and 100 $\mu$M-6-methyltetrahydropterine (6-MPH$_4$), the latter being one of the more commonly used synthetic analogues of the naturally occurring co-factor (Shiman, Akino & Kaufman, 1971), and 100 $\mu$M being the optimum level of 6-MPH$_4$ established for catecholaminergic structures in brain (Patrick & Barchas, 1976). Control carotid bodies were incubated under similar conditions but without 6-MPH$_4$, and the results of these experiments are shown in Table 1. Synthesis of both [$^3$H]DA and [$^3$H]NA in the carotid body was markedly increased ($P < 0·01$) in the presence of co-factor. This effect was less pronounced when similar experiments were performed with the superior cervical ganglion (Table 1), suggesting that the carotid body by comparison has limited stores of co-factor. This agrees with the recent observations of I. Hanbauer (personal communication) that co-factor concentrations in rat carotid body are extremely low, probably reflecting a rapid turnover and a small endogenous pool of this substance in the tissue. These findings may not be altogether surprising in view of the high rate of blood flow in the carotid body and the possible dependence of peripheral catecholaminergic structures on regional blood flow for delivery of the active form of co-factor produced in the liver (Gal, Hanson & Sherman, 1976). In all subsequent experiments, the incubation media contained 100 $\mu$M-6-MPH$_4$.

*Time course of synthesis.* Carotid bodies were incubated in media containing a concentration of [$^3$H]tyrosine (40 $\mu$M) similar to that present in rabbit plasma (Reddy & Kinsey, 1964). As shown in Fig. 2*A*, labelled tyrosine levels in the tissue approached equilibrium with the medium in 30 min, at which time the tissue concentration of [$^3$H]tyrosine could be calculated to be approximately 65 $\mu$M (assuming 56% extracellular space from [$^{14}$C]inulin wash-out experiments; S. Fidone & C. Gonzalez, unpublished observations). This concentration is not unlike the value of 50 $\mu$M reported for rabbit brain and very similar to the level of tyrosine measured for ox carotid body (69 $\mu$M; Harms, 1979). Fig. 2*A* also shows that the synthesis of [$^3$H]DA

and [$^3$H]NA was linear for incubation periods up to 4 hr. Tritiated catecholamines were not detected in the incubation media when [$^3$H]tyrosine was used as precursor, a finding consistent with that observed for catecholamine synthesis from tyrosine in other systems (Levitt *et al.* 1965). The turnover times of DA and NA synthesized from tyrosine in rabbit carotid body are 16·2 and 10·4 hr, respectively, determined in the same manner as for DOPA.

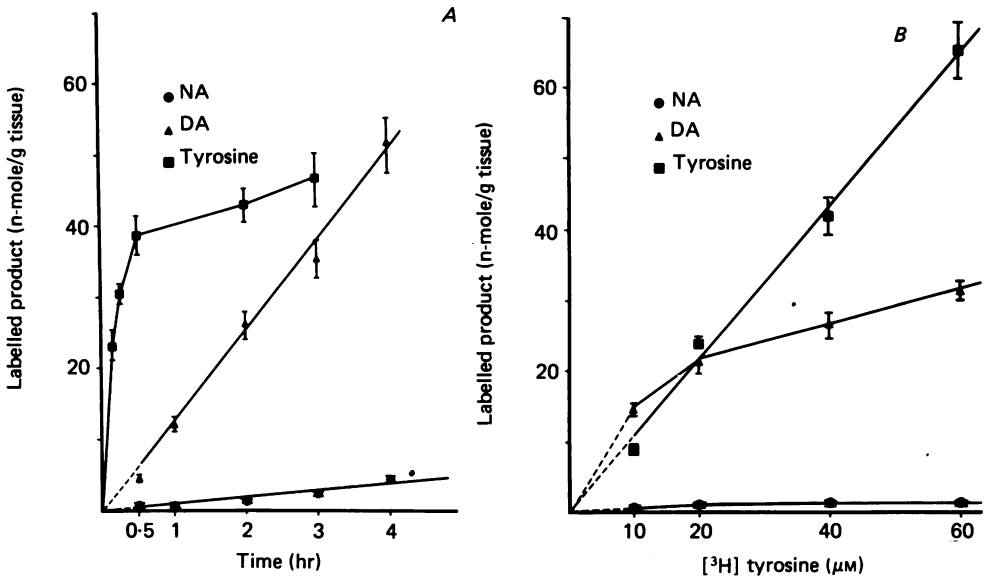


Fig. 2. *A*, time course of synthesis for [$^3$H]DA and [$^3$H]NA in rabbit carotid body using [$^3$H]tyrosine (40 μM) as precursor. For [$^3$H]DA, $r = 0{\cdot}99$; for [$^3$H]NA, $r = 0{\cdot}97$. *B*, relationship between [$^3$H]tyrosine concentration and tissue levels of [$^3$H]DA, [$^3$H]NA and [$^3$H]tyrosine in rabbit carotid bodies incubated for 2 hr with the precursor compound.

*$K_m$ for DA and NA synthesis.* In order to establish the kinetics of the over-all process of synthesis and storage of catecholamines in rabbit carotid body, tissue samples were incubated for 2 hr with concentrations of [$^3$H]tyrosine varying from 10 to 60 μM. Fig. 2*B* shows the levels of [$^3$H]DA, [$^3$H]NA and [$^3$H]tyrosine present in the tissues at the end of the incubation periods as a function of the concentration of [$^3$H]tyrosine in the media (media concentration of [$^3$H]tyrosine was unchanged during period of incubation). [$^3$H]DA and [$^3$H]NA synthesis exhibited the characteristics of saturable processes, whereas the accumulation of non-metabolized [$^3$H]tyrosine remained linear ($r = 0{\cdot}99$) throughout the concentration range. When the data for [$^3$H]DA and [$^3$H]NA were plotted on a double-reciprocal format (Fig. 3), it was found that the over-all $K_m$ for catecholamine synthesis from tyrosine in rabbit carotid body was approximately 17 μM ($K_m$ for DA = 16·8 μM; $K_m$ for NA = 17·6 μM).

*Effects of sympathectomy and deafferentation.* Sympathectomized and carotid sinus nerve-denervated carotid bodies and their contralateral normally innervated controls were incubated for 3 hr in 40 μM-[$^3$H]tyrosine. As shown in Table 2, sympathectomy

reduced by 80% ($P < 0.001$) the level of [$^3$H]NA synthesized by the carotid body, which is similar to the reduction described earlier for sympathectomized carotid bodies incubated with [$^3$H]DOPA. In contrast, [$^3$H]DA and [$^3$H]tyrosine levels in the tissue were unaltered following sympathectomy. Carotid sinus nerve denervation likewise did not alter [$^3$H]DA, [$^3$H]NA or [$^3$H]tyrosine concentrations in the tissues.

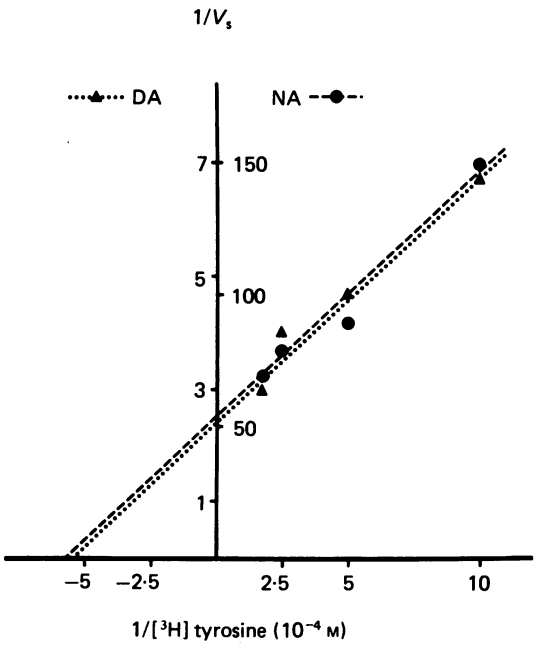


Fig. 3. Double-reciprocal plot of the data for [$^3$H]DA and [$^3$H]NA shown in Fig. 2*B*. Velocity ($V_s$) is expressed as n-mole $\times 10^{-2}$ [$^3$H]DA or [$^3$H]NA synthesized per g tissue in 2 hr. $K_m$ for DA = 16·9 $\mu$M; $K_m$ for NA = 17·6 $\mu$M.

## DISCUSSION

Studies in other catecholaminergic systems have compared the relative utilization of the precursors DOPA and tyrosine as a means of characterizing the rate-limiting and regulatory steps in catecholamine synthesis (Levitt *et al.* 1965; Roth, Stjarne & von Euler, 1966; Sedvall & Kopin, 1967; Weiner & Rabadjija, 1968; Thoa, Johnson, Kopin & Weiner, 1971; Murrin & Roth, 1976). The experiments reported here on unstimulated rabbit carotid bodies have examined the biosynthesis of [$^3$H]catecholamines from the labelled form of these precursors. The results demonstrate that the carotid body is similar to other catecholaminergic tissues in respect to the kinetics of DA and NA synthesis.

*Studies with [$^3$H]DOPA as precursor.* When [$^3$H]DOPA was used as precursor, the carotid body synthesized large amounts of [$^3$H]catecholamines, which probably exceeded the capacity of the tissue to store these biogenic amines and consequently resulted in the liberation of these substances into the medium. This high rate of

synthesis is characteristic of catecholaminergic tissues when the rate-limiting step, hydroxylation of tyrosine, is obviated (Levitt *et al.* 1965; Persson, 1969; Nagatsu, 1973). Immunohistochemical studies in rat carotid body have demonstrated an immunoprecipitate for DOPA-decarboxylase (L-aromatic amino acid decarboxylase) which exceeds that obtained for tyrosine hydroxylase (Bolme, Fuxe, Hokfelt &

TABLE 2. Effects of denervations on [$^3$H]catecholamine synthesis from [$^3$H]tyrosine in rabbit carotid body

| Condition | [$^3$H]tyrosine | [$^3$H]DA | [$^3$H]NA |
|---|---|---|---|
| Control | 46·09 ± 3·77 | 33·90 ± 2·59 | 2·68 ± 0·48 |
| Sinus nerve cut | 43·03 ± 3·06 | 35·30 ± 1·50 | 3·14 ± 0·51 |
| Sympathectomy | 44·52 ± 3·46 | 35·00 ± 3·2 | 0·53 ± 0·07* |

Values are expressed as n-mole/g tissue (± S.E.). Sham operations and removal of the carotid sinus nerve or the superior cervical ganglion were performed 12–15 days before the experiments. Carotid bodies were incubated for 3 hr in 40 $\mu$M-[$^3$H]tyrosine.

* $P < 0{\cdot}01$.

Goldstein, 1977); the prevalence of DOPA-decarboxylase is a common property of catecholaminergic tissues (Molinoff & Axelrod, 1971). Also, the relationship between precursor concentration and [$^3$H]DA and [$^3$H]NA synthesis was nearly linear throughout the concentration range, 10–40 $\mu$M-[$^3$H]DOPA, which likewise agrees with studies from other catecholamine-containing structures (Levitt *et al.* 1965), and may be attributed to the high $K_m$ of DOPA-decarboxylase (Lovenberg, Weissbach & Udenfriend, 1962; Dairman, Christensen & Udenfriend, 1973).

The ratios of [$^3$H]DA/[$^3$H]NA (3–6; see Fig. 1) found in the carotid body when [$^3$H]DOPA is used as precursor are much lower than that predictable from the endogenous DA and NA levels reported for rabbit carotid body (DA/NA ratio; 13–27; Dearnaley *et al.* 1968). This finding was not surprising, however, since noradrenergic tissues have been shown to utilize DOPA as precursor more efficiently than dopaminergic structures (Udenfriend *et al.* 1966; Persson, 1969). Thus, the amount of [$^3$H]NA present in the sympathetic nerve endings plus the NA-containing type I cells (Verna, 1975) should be greater than normal, relative to [$^3$H]DA, when [$^3$H]DOPA is used as precursor. When [$^3$H]tyrosine was used as precursor in our experiments, the [$^3$H]DA/[$^3$H]NA ratio was approximately 13, which is within the range reported for these endogenous catecholamines in rabbit carotid body (Dearnaley *et al.* 1968).

Carotid bodies which had been sympathectomized 12–15 days before synthesized one tenth of the [$^3$H]NA and approximately two thirds of the [$^3$H]DA synthesized by control carotid bodies when [$^3$H]DOPA was used as precursor. The decrease in [$^3$H]DA, which was not observed when [$^3$H]tyrosine was used as precursor (Table 2), can be attributed to the formation of DA pools in NA-containing sympathetic terminals. Normally very low, DA levels in noradrenergic neurones are increased many-fold in the presence of DOPA (Persson, 1969; Jonsson & Sachs, 1970).

*Studies with [$^3$H]tyrosine as precursor.* The $K_m$ of 17 $\mu$M for biosynthesis of [$^3$H]DA and [$^3$H]NA from [$^3$H]tyrosine in the carotid body agrees with that reported for other catecholaminergic tissues (20 $\mu$M; Levitt *et al.* 1965), and is similar to the $K_m$ for purified tyrosine hydroxylase (10 $\mu$M; Nagatsu *et al.* 1964).

The estimated turnover times for DA and NA in rabbit carotid body were found to be 16·2 and 10·4 hr, respectively. This value for DA turnover is the same as that of 16 hr found by Helpap & Hempel (1968) for rat carotid body. However, Hanbauer & Hellstrom (1978) have reported much shorter turnover times for DA in rat carotid body, namely 2–3 hr, but this time should be too short to allow for complete turnover of endogenous DA in rat carotid body (30 p-mole/pair carotid body; Hellstrom, Hanbauer & Costa, 1976) if one assumes the tyrosine hydroxylase (TH) activity (3·7–4·9 p-mole catecholamines/pair carotid body per hr) reported by Hanbauer, Lovenberg & Costa (1977). In our studies with rat carotid body, however, we observed much higher TH activities in this organ (5–6 p-mole/mg tissue per hr; Gonzalez, Kwok, Gibb & Fidone, 1979); thus, the issue of turnover time for DA in rat carotid body remains unsettled at this time. It is important to note here that the method we employed to estimate turnover of catecholamines in the rabbit carotid body, when applied to the rat striatum (Gonzalez, Obeso & Fidone, 1979) yields values which are in accord with those reported in the literature (see Bacopoulos & Bhatnagar, 1977).

The results of our experiments using [$^3$H]tyrosine as precursor with denervated carotid bodies suggest that all the DA stores and at least 20% of the NA stores are contained in the parenchymal cells of the organ. The remaining NA is localized to the sympathetic innervation to this tissue. These data for the rabbit agree qualitatively with the 50% reduction in NA in sympathectomized rat carotid bodies (Hanbauer & Hellstrom, 1978) and the 47% decrease in dopamine $\beta$-hydroxylase activity in sympathectomized cat carotid bodies (Belmonte, Gonzalez & Garcia, 1977). In contrast to the effects of sympathectomy, transection of the carotid sinus nerve did not significantly alter either [$^3$H]DA or [$^3$H]NA levels in the rabbit carotid body.

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## REFERENCES

BACOPOULOS, N. G. & BHATNAGAR, R. K. (1977). Correlation between tyrosine hydroxylase activity and catecholamine concentration or turnover in brain regions. *J. Neurochem.* **29**, 639–643.

BARON, M. & EYZAGUIRRE, C. (1977). Effects of temperature on some membrane characteristics of carotid body cells. *Am. J. Physiol.* **233**, 35–46.

BELMONTE, C., GONZALEZ, C. & GARCIA, A. (1977). Dopamine beta-hydroxylase activity in cat carotid body. In *Chemoreception in the Carotid Body*, ed. ACKER, H., FIDONE, S., PALLOT, D., EYZAGUIRRE, C., LUBBERS, D. W. & TORRANCE, R. W., pp. 99–105. Berlin: Springer-Verlag.

BESSON, M. J., CHERAMY, A., FELTZ, P. & GLOWINSKI, J. (1969). Release of newly synthesized dopamine from dopamine-containing terminals in the striatum of the rat. *Proc. natn. Acad. Sci. U.S.A.* **62**, 741–748.

BLACK, A. M. S., COMROE, J. H., JR. & JACOBS, L. (1972). Species differences in carotid body response of cat and dog to dopamine and serotonin. *Am. J. Physiol.* **223**, 1097–1102.

BOLME, P., FUXE, K., HOKFELT, T. & GOLDSTEIN, M. (1977). Studies on the role of dopamine in cardiovascular and respiratory control: central *versus* peripheral mechanisms. In *Advances in Biochemical Psychopharmacology*, vol. 16, ed. COSTA, E. & GESSO, G. L. New York: Raven Press.

CHIOCCHIO, S. R., BISCARDI, A. M. & TRAMEZZANI, J. H. (1966). Catecholamines in the carotid body of the cat. *Nature, Lond.*, **212**, 834–835.

CHIOCCHIO, S. R., KING, M. P. & ANGELAKOS, E. T. (1971). Carotid body catecholamines: histochemical studies on the effects of drug treatments. *Histochemistry*, **25**, 52–59.

CROUT, J. R. (1961). Catecholamines in urine. In *Standard Methods of Clinical Chemistry*, ed. SELIGSON, D., vol. 3, p. 62. New York: Academic Press.

DAIRMAN, W., CHRISTENSON, J. & UDENFRIEND, S. (1973). Characterization of dopa decarboxylase. In *Frontiers in Catecholamine Research*, ed. USDIN, E. & SNYDER, S., pp. 61–67. London: Pergamon Press.

DEARNALEY, D. P., FILLENZ, M. & WOODS, R. I. (1968). The identification of dopamine in the rabbit's carotid body. *Proc. R. Soc.* B **170**, 195–203.

FILLENZ, M. (1975). The function of the Type I cells of the carotid body. In *The Peripheral Arterial Chemoreceptors*, ed. PURVES, M. J., pp. 133–143. Cambridge: Cambridge University Press.

GAL, E. M., HANSON, G. & SHERMAN, A. (1976). Biopterin. I. Profile and quantification in rat brain. *Neurochem. Res.* **1**, 511–523.

GONZALEZ, C., KWOK, Y., GIBB, J. & FIDONE, S. (1979). Effects of hypoxia on tyrosine hydroxylase activity in rat carotid body. *J. Neurochem.* **33**, 713–719.

GONZALEZ, C., KWOK, Y., GIBB, J. & FIDONE, S. (1981). Physiological and pharmacologic effects on TH activity in rabbit and cat carotid body. *Am. J. Physiol.* **240** (Reg., Int. & Comp. Physiol. 9): R38–R43.

GONZALEZ, C., OBESO, A. & FIDONE, S. (1979). Tris buffer: effects on catecholamine synthesis. *J. Neurochem.* **32**, 1143–1145.

HANBAUER, I. & HELLSTROM, S. (1978). The regulation of dopamine and noradrenaline in the rat carotid body and its modification by denervation and by hypoxia. *J. Physiol.* **282**, 21–34.

HANBAUER, I., LOVENBERG, W. & COSTA, E. (1977). Induction of tyrosine 3-mono-oxygenase in carotid body of rats exposed to hypoxic conditions. *Neuropharmacology*, **16**, 277–282.

HARMS, B. (1979). Gehalte an freien Aminosäuren, biogenen Aminen und Neurotransmittern und Untersuchungen zur Catecholamin-Biosynthese im Glomus Caroticum des Rindes. Doctoral dissertation, University of Hamburg.

HELLSTROM, S., HANBAUER, I. & COSTA, E. (1976). Selective decrease in dopamine content in rat carotid body during exposire to hypoxic conditions. *Brain Res.* **118**, 352–355.

HELLSTROM, S. & KOSLOW, S. H. (1975). Biogenic amines in carotid body of adult and infant rats, a gas chromatographic–mass spectrometric assay. *Acta physiol. scand.* **93**, 540–547.

HELPAP, B. & HEMPEL, K. (1968). Autoradiographische Untersuchungen mit $^3$H-Dopa zum Catecholamin-Stoffwechsel des Karotiskorperchens. *Verh. dt. Ges. Pathol.* **52**, 464–468.

HILDEBRAND, J. G., BARKER, D. L., HERBERT, E. & KRAVITZ, E. A. (1971). Screening for neurotransmitters: a rapid radiochemical procedure. *J. Neurobiol.* **2**, 231–246.

JONSSON, G. & SACHS, C. (1970). Synthesis of noradrenaline and 3,4-dihydroxyphenylalanine (DOPA) and dopamine in adrenergic nerves of mouse atrium – effect of reserpine, monoamine oxidase and tyrosine hydroxylase inhibition. *Acta physiol. scand.* **80**, 307–322.

KETTLER, R., BARTHOLINI, G. & PLETSCHER, A. (1974). *In vivo* enhancement of tyrosine hydroxylation in rat striatum by tetrahydrobiopterin. *Nature, Lond.* **249**, 476–478.

LEVER, J. D. & BOYD, J. D. (1957). Osmiophile granules in the glomus cells of the rabbit carotid body. *Nature, Lond.* **179**, 1082–1083.

LEVITT, M., SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1965). Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. *J. Pharmac. exp. Ther.* **148**, 1–8.

LLADOS, F. & ZAPATA, P. (1978*a*). Effects of dopamine analogues and antagonists on carotid body chemosensors *in situ*. *J. Physiol.* **274**, 487–499.

LLADOS, F. & ZAPATA, P. (1978*b*). Effects of adrenoceptor stimulating and blocking agents on carotid body chemosensory inhibition. *J. Physiol.* **274**, 501–509.

LOVENBERG, W., WEISSBACH, H. & UDENFRIEND, S. (1962). Aromatic L-amino acid decarboxylase. *J. biol. Chem.* **237**, 89–93.

MOLINOFF, P. & AXELROD, J. (1971). Biochemistry of catecholamines. *A. Rev. Biochem.* **40**, 465–500.

MURRIN, L. C. & ROTH, R. H. (1976). Dopaminergic neurons: effects of electrical stimulation on dopamine biosynthesis. *Molec. Pharmacol.* **12**, 463–475.

NAGATSU, T. (1973). *Biochemistry of Catecholamines*. Baltimore: University Park Press.

NAGATSU, T., LEVITT, M. & UDENFRIEND, S. (1964). Tyrosine hydroxylase: the initial step in norepinephrine biosynthesis. *J. biol. Chem.* **239**, 2910–2917.

PATRICK, R. L. & BARCHAS, J. D. (1976). Dopamine synthesis in rat brain striatal synaptosomes. II. Dibutyrylcyclic adenosine 3′: 5′-monophosphoric acid and and 6-methyltetrahydropterine-

induced synthesis increases without an increase in endogenous dopamine release. *J. Pharmac. exp. Ther.* **197**, 97–104.

PERSSON, T. (1969). Central and peripheral catecholamine turnover studied by means of $^3$H-dopa and $^3$H-tyrosine. *Acta pharmac. tox.* **27**, 397–409.

REDDY, D. V. N. & KINSEY, V. E. (1964). Chemistry and dynamics of aqueous humor. In *The Rabbit in Eye Research*, ed. PRINCE, J. H., pp. 218–319. Springfield, IL: Charles C. Thomas.

ROTH, R. H., STJARNE, L. & EULER, U. S. VON (1966). Acceleration of noradrenaline biosynthesis by nerve stimulation. *Life Sci., Oxford*, **5**, 1071–1075.

SAMPSON, S. R., AMINOFF, M. J., JAFFE, R. A. & VIDRUK, E. H. (1976). Analysis of inhibitory effect of dopamine on carotid body chemoreceptors in cats. *Am. J. Physiol.* **230**, 1494–1498.

SEDVALL, G. C. & KOPIN, I. J. (1967). Acceleration of norepinephrine synthesis in the rat submaxillary gland *in vivo* during sympathetic nerve stimulation. *Life Sci., Oxford*, **6**, 45–51.

SHIMAN, R., AKINO, M. & KAUFMAN, S. (1971). Solubilization and partial purification of tyrosine hydroxylase from bovine adrenal medulla. *J. biol. Chem.* **246**, 1330–1340.

THOA, N. B., JOHNSON, D. J., KOPIN, I. J. & WEINER, N. (1971). Acceleration of catecholamine formation in the guinea-pig vas deferens after hypogastric nerve stimulation: roles of tyrosine hydroxylase and new protein synthesis. *J. Pharmac. exp. Ther.* **178**, 442–449.

UDENFRIEND, S., ZALTZMAN-NIRENBERG, P. & NAGATSU, T. (1965). Inhibitors of purified beef adrenal tyrosine hydroxylase. *Biochem. Pharmacol.* **14**, 837–845.

UDENFRIEND, S., ZALTZMAN-NIRENBERG, P., GORDON, R. & SPECTOR, S. (1966). Evaluation of the biochemical effects produced *in vivo* by inhibitors of the three enzymes involved in norepinephrine biosynthesis. *Mol. Pharmacol.* **2**, 95–105.

VERNA, A. (1975). Contribution a l'Étude du Glomus Carotidien du Lapin. Recherches Cytologiques, Cytochimimiques et Expérimentales. Doctoral thesis, Bordeaux.

WEINER, N. & RABADJIJA, M. (1968). The effect of nerve stimulation on the synthesis and metabolism of norepinephrine in the isolated guinea-pig hypogastric nerve–vas deferens preparation. *J. Pharmac. exp. Ther.* **160**, 61–71.

ZAPATA, P. (1975). Effects of dopamine on carotid chemo- and baroreceptors *in vitro*. *J. Physiol.* **244**, 235–257.

ZAPATA, P., HESS, A., BLISS, E. L. & EYZAGUIRRE, C. (1969). Chemical, electron microscopic and physiological observations on the role of catecholamines in the carotid body. *Brain Res.* **14**, 473–496.