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Inhibition of endogenous catecholamine biosynthesis by 3-iodo-L-tyrosine

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THE enzyme, tyrosine hydroxylase, which catalyzes the initial step in the formation of norepinephrine from the dietary precursor tyrosine, has been recently isolated and partially purified from the adrenal medulla.[1] Several aromatic L-amino acids were found to be effective inhibitors of tyrosine hydroxylase *in vitro*.[1] The inhibition *in vivo* of tyrosine hydroxylase by α-methyltyrosine has been described.[2] It was recently shown that 3-iodo-L-tyrosine is a potent inhibitor of tyrosine hydroxylase *in vitro*,[3,4] and that the inhibition is of a competitive nature. The present study shows that 3-iodo-L-tyrosine does lower endogenous levels of catecholamines in various organs. The decrease in the catecholamine levels is primarily due to the inhibition of catecholamine synthesis at the tyrosine hydroxylase stage.

Rats were treated at various time intervals with 3-iodo-L-tyrosine; solutions for injections were prepared by dissolving the substance with 0·1 N HCl. The pH was then readjusted to 4·5 to 5·5 by addition of 0·1 N NaOH immediately before i.p. injection. The animals were killed at various time intervals and the tissues removed rapidly and immediately analyzed or stored in the freezer. The hearts, brains, and salivary glands were homogenized in cold 0·4 N perchloric acid. After centrifugation, the supernatant fluid was adjusted to pH 5 with K_2CO_3, and the precipitated potassium perchlorate was removed by filtration. The catecholamines were adsorbed on alumina and then eluted with 0·2 N acetic acid. Norepinephrine and dopamine were determined by fluorometric method.[5, 6] The adrenal glands were homogenized in 3% of trichloroacetic acid and the excess of the trichloroacetic acid removed by extraction with ether. The aqueous extract was adjusted to pH 5·5, and the total catecholamines were determined fluormetrically.[5]

The data in Table 1 show that 4 hr after the administration of 200 mg 3-iodo-L-tyrosine/kg the levels of catecholamines were decreased in all analyzed organs. Brain catecholamines were reduced to a greater extent than the catecholamines in the heart or salivary glands. The total catecholamines in the adrenal glands were only slightly reduced. After repeated administration of 3-iodo-L-tyrosine, the levels of the catecholamines in the brain were again reduced to a greater extent than in the other analyzed organs, but the difference was less apparent than after single-dose administration. It is conceivable that the pronounced reduction of catecholamines in the brain after a single administration of 3-iodo-L-tyrosine is due to the rapid turnover of the catecholamines in the central nervous system. The minimal changes in the adrenal catecholamine levels may also be due to the slow turnover of the catecholamines in these organs. The concentration of the inhibitor in different tissues may also affect the degree of the inhibition.

In separate experiments we investigated whether 3-iodo-L-tyrosine induces release of norepinephrine-^{3}H or prevents its uptake by the heart. It is evident from the data presented in Table 2 that a single dose of 200 mg 3-iodo-L-tyrosine/kg produces no significant release of norepinephrine-^{3}H from the heart. Table 2 also shows that repeated administration of 3-iodo-L-tyrosine slightly prevents the uptake in the heart of exogenous norepinephrine-^{3}H. Since the endogenous levels of norepinephrine in the heart of animals treated with 3-iodo-L-tyrosine are reduced to a much greater extent than are the exogenous norepinephrine-^{3}H levels (Table 2), it can be concluded that the decrease in the catecholamine levels is mainly due to the inhibition of catecholamine biosynthesis.

TABLE 1. CATECHOLAMINE CONTENT IN RAT TISSUES AFTER A SINGLE OR MULTIPLE DOSE OF 3-IODO-L-TYROSINE

Tissue	Norepinephrine (μg/g)		
	Control	3-iodo-L-tyrosine	
		a	*b*
Brain	0·40 ± 0·06 (0·70 ± 0·10)	0 15 ± 0 02 (0·25 ± 0·05)	0·10 ± 0·02 (0·15 ± 0·05)
Heart	0·65 ± 0·10	0·40 ± 0·05	0 30 ± 0 05
Salivary gland	0·80 ± 0·10	0·65 ± 0 1	0 50 ± 0 07
Adrenal gland*	950 ± 90	910 ± 90	840 ± 90

The results represent averages of three experiments.

Column *a* represents the levels of catecholamines after administration of a single dose of 3-iodo-L-tyrosine (200 mg/kg i.p); *b* represents the levels of catecholamines following repeated administration of 3-iodo-L-tyrosine (80 mg/kg i.p. was administered every 3 hr for 9 hr and the animals were killed 3 hr after the last dose).

The results in parentheses represent the brain dopamine levels.

* In the adrenal glands the total catecholamines (norepinephrine plus epinephrine) were measured.

TABLE 2. EFFECT OF 3-IODO-L-TYROSINE ON THE EXOGENOUS AND ENDOGENOUS NOREPINEPHRINE CONTENT IN THE HEART

Exp. no.	^{3}H-Norepinephrine (mμc/g)	Norepinephrine (μg/g)	% Change	
			^{3}H-Norepinephrine	Norepinephrine
1	550 ± 50	0·68 ± 0·10		
2	500 ± 50	0·48 ± 0·07	10	30
3	460 ± 40	0·30 ± 0·05	15	56

Results represent averages of two experiments.

Experiment 1 served as control. Rats were given 20 μc ^{3}H-norepinephrine i.v., and killed 3 hr later.

In experiment 2, rats were given 20 μc of ^{3}H-norepinephrine i.v., followed 30 min later by 200 mg 3-iodo-L-tyrosine kg i.p. The animals were killed 3 hr after administration of ^{3}H-norepinephrine.

In experiment 3, rats were given three times every 3 hr 80 mg 3-iodo-L-tyrosine kg i.p. Thirty min before the last injection, the animals received 20 μc ^{3}H-norepinephrine i.v. The animals were killed 3 hr after administration of ^{3}H-norepinephrine.

The present results confirm the previously reported findings[2] that the inhibition of tyrosine hydroxylase *in vivo* results in a lowering of tissue catecholamine levels. α-Methyltyrosine and 3-iodo-L-tyrosine are competitive inhibitors of tyrosine hydroxylase, and they both lower endogenous catecholamine tissue levels. The inhibition of dopamine-β-hydroxylase by disulfiram results also in a lowering of tissue norepinephrine levels.[7, 8] However, the inhibition of tyrosine hydroxylase may produce different pharmacological effects than does the inhibition of dopamine-β-hydroxylase. The inhibition of tyrosine hydroxylase results in a lowering of both tissue catecholamines—dopamine and norepinephrine—while the inhibition of dopamine-β-hydroxylase results in a lowering of norepinephrine tissue levels and an increase of dopamine tissue levels.[9]

The nature of the association between thyroid action and the sympathetic nervous system has been the subject of many investigations.[10, 11] It has been shown that thyroxine inhibits monoamine oxidase activity in the liver,[12] and that thyroxine and other thyroid hormones inhibit catechol methyl transferase *in vitro* and *in vivo*.[13, 14] Some controversy exists concerning the nature of organic iodine in peripheral plasma. Although most investigators have found that the major iodinated compounds are

thyroxine and triiodothyronine,[15] some have also reported the presence of iodotyrosine in the peripheral blood of thyrotoxic or normal humans, or those stimulated by thyrotropin.[16] The present findings that 3-iodo-L-tyrosine lowers the catecholamine levels *in vivo* raises the question whether thyroid hormones affect the biosynthesis of catecholamines *in vivo*.

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Action of angiotensin on myocardial and renal catecholamines in the rabbit

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THERE have been several recent reports indicating the necessity of an intact sympathetic nervous system for the cardiovascular action of angiotensin.[1–4] Benelli *et al.*[2] have reported the involvement of the peripheral sympathetic nerves and have hypothesized that angiotensin acts at the peripheral nerve endings by promoting a greater output of norepinephrine. There have also been suggestions that angiotensin has a specific storage pool or might exchange with or replace norepinephrine stores in tissues.[3] On the other hand, Zimmerman *et al.*[4] have reported no definite indication that the release of norepinephrine during nerve stimulation was facilitated by angiotensin.

Regarding the action of angiotensin on the heart, Youmans *et al.*[5] observed that the positive chronotropic effect of angiotensin in the ganglionically blocked dog was inhibited by bretylium. These authors conclude that this effect of angiotensin is dependent on catecholamines. In the studies of Gross and co-workers,[6] reserpine pretreatment for 3 days greatly diminished the positive inotropic effect of angiotensin in rats as evaluated by its effect on cardiac output.

Fowler and Holmes,[7] on the other hand, observed that reserpine treatment did not change the positive inotropic effect of angiotensin. Similar results have been reported by Koch-Weser[8] on the isolated cat papillary muscle. There is good evidence that angiotensin is a potent releaser of catecholamines from the adrenal medulla.[9–10]