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MONOAMINE OXIDASE ACTIVITY OF PERIPHERAL ORGANS AND SYMPATHETIC GANGLIA OF THE RAT AFTER IMMUNOSYMPATHECTOMY*

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Abstract—Treatment of litter-mate rats with the antiserum to the mouse submaxillary gland nerve growth factor of Levi-Montalcini and Cohen resulted in significant decreases in monoamine oxidase activity of the superior cervical, stellate, and thoracic chain ganglia, the spleen, submaxillary glands, kidneys, and liver. The monoamine oxidase activity of the retinas, pineal gland, pituitary gland, lungs, atria, and uterus showed small but statistically insignificant decreases. The monoamine oxidase activity of the ventricles, various portions of the stomach, and proximal and distal small intestine was not affected by treatment with the antiserum. It is postulated that a relation can be shown between sympathetic innervation and monoamine oxidase activity. This can be shown only in those tissues in which the monoamine oxidase associated with sympathetic function constitutes an appreciable portion of the total monoamine oxidase activity. In most of the organs and tissues studied, only a small portion of the monoamine oxidase activity is associated with the nerve endings of the sympathetic postganglionic fibers, whereas the larger portion of the total activity is associated with nonadrenergic sites. Monoamine oxidase activity was measured by the method of Lovenberg and co-workers (*J. Pharmac. exp. Ther.*, 1962), with slight modifications found to give reproducible, quantitative results (Klingman and Klingman, *Biochem. Pharmac.*, 1966).

MONOAMINE OXIDASE has been implicated as the catabolic enzyme of a portion of intraneuronally-released norepinephrine. The mouse submaxillary gland nerve growth factor (NGF) of Levi-Montalcini and Cohen and the antiserum to the NGF[1–4] present useful tools for studying the relation between the extent of sympathetic innervation (near-absence after antiserum treatment; normal and excessive after NGF administration), the functional integrity of the sympathetic nervous system, the localization and metabolism of catecholamines, and the activities of the anabolic and catabolic enzymes of catecholamine metabolism. The effects of immunosympathectomy on dopa-decarboxylase activity and catecholamine concentrations of peripheral organs have been previously reported.[5] Levi-Montalcini and Angeletti[6] described large

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decreases (up to 70 per cent of control values) in monoamine oxidase activity of the spleen, heart, and submaxillary glands after the administration of the antiserum to the NGF. These data indicate that up to 70 per cent of monoamine oxidase activity in crude tissue homogenates from these organs is associated with sympathetic fibers and function.

The effects of surgical postganglionic denervation or ganglionectomy on monoamine oxidase activity of peripheral organs and tissues are not clear-cut. Of the denervated tissues (nictitating membrane,[7] iris, and foreleg arteries[8] of the cat, and arteries of the rabbit ear[9]), only the monoamine oxidase activity of the cat's nictitating membrane showed a decrease. Potter *et al.*[10] reported a small reduction in the denervated dog heart (autotransplants). Snyder *et al.*[11] reported a 50 per cent reduction of monoamine oxidase activity in rat pineal glands after bilateral superior cervical ganglionectomies. The results of this study conflict with those of Håkanson and Owman,[12] who found no change in the monoamine oxidase activity under similar conditions.

METHODS

Animals. Sprague-Dawley rats of both sexes, born and raised in this laboratory, were used in the investigation. Each litter of newborn animals was divided into two groups: controls and those treated by s.c. injection daily of 300 units antiserum/g, corresponding to 1·56 mg lyophilized serum/g/day, for 7 days; the first dose was given 12 to 18 hr after birth. The animals were sacrificed, 2–3·5 months and 7–8·5 months after completion of the antiserum treatment, by decapitation after pentobarbital sodium anesthesia (30 to 40 mg/kg, i.p.).

Analytical procedure. Tissues used for the determination of monoamine oxidase activity were rapidly dissected in the cold and immediately frozen. The thawed, but still cold, tissues were homogenized in cold 0·25 M sucrose. Since control and antiserum-treated litter-mates were available, paired experiments were conducted, and three or four pairs of tissues were incubated together. Monoamine oxidase activity was measured by the method of Lovenberg *et al.*,[13] with slight modifications found to give reproducible, quantitative results. An extensive study of monoamine oxidase activity in tissues from control rats, utilizing this method, has been reported.[14]

The incubation mixture consisted of 0·3 ml homogenate (in 0·25 M sucrose), 0·5 ml 0·25 M phosphate buffer (pH 7·3; 125 μmoles), 0·1 ml nicotinamide (30 μmoles), 0·1 ml NAD (7 μmoles), 0·3 ml distilled water and 0·1 ml of either a crude preparation of guinea pig kidney aldehyde dehydrogenase or water. After a preincubation period of 5 min, the reaction was initiated by the addition of 0·1 ml tryptamine (3·5 μmoles). After mixing, two 0·5-ml aliquots were immediately transferred to preincubated tubes and incubated for the appropriate time period. The final 0·5 ml of the mixture was used as the 0-time sample (or tissue blank). In each assay, the third aliquot was used either for measurement of a second time period (to assure linearity) or for addition of the monoamine oxidase inhibitor, β-phenylisopropylhydrazine (Catron, JB-516),* 10^{-3} M (to assure specificity). The reaction was terminated by the addition of 0·4 ml of 2 N HCl, and the tubes were kept in crushed ice or frozen, dependent upon the time elapsing before extraction. For the extraction of indoleacetic acid, 7 ml of washed

* The author wishes to thank Dr. Murray Finkelstein of Lakeside Laboratories for the generous supply of Catron.

toluene* was added, and the tube agitated several times on a Vortex mixer. After centrifugation, 5 ml of the toluene was transferred to a tube containing 1·5 ml of 0·25 M phosphate buffer (pH 7·3), agitated in a mechanical shaker, and centrifuged. The fluorescence of the indoleacetic acid was then measured in an aliquot of the aqueous phase (phosphate buffer) with an Aminco-Bowman spectrophotofluorometer at 290/370 mμ (uncorrected wavelengths). To increase the recovery of indoleacetic acid, two toluene extractions were performed on the incubation aliquots from some tissues, and the indoleacetic acid from the pooled toluene fractions was partitioned into the phosphate buffer. The protein content of the tissues was determined by the method of Lowry *et al.*[15]

The monoamine oxidase activity, in the absence of exogenous aldehyde dehydrogenase, was measured in paired tissues from one series of antiserum-treated and control litter-mates. Another such series was used to measure the monoamine oxidase activity with added aldehyde dehydrogenase, except in two cases in which the tissues of a single antiserum-treated and of a single control rat were incubated with and without exogenous aldehyde dehydrogenase.

RESULTS

Significant decreases in ganglionic monoamine oxidase activity were noted in immunosympathectomized rats. The greatest decrease occurred in the superior cervical ganglia; less than 10 per cent of monoamine oxidase activity remained after antiserum treatment (Table 1). In stellate ganglia and thoracic chains, 15 to 18 per

TABLE 1. MONOAMINE OXIDASE ACTIVITY IN SYMPATHETIC GANGLIA, RETINA, PINEAL GLAND, AND PITUITARY GLAND OF CONTROL AND ANTISERUM-TREATED RATS IN THE PRESENCE AND ABSENCE OF EXOGENOUS ALDEHYDE DEHYDROGENASE (AD)

Tissue	Control rats		Antiserum-treated rats	
	No AD (5 rats) 264 ± 25*; 4♀, 1♂	With AD (7 rats) 341 ± 49; 4♀, 3♂	No AD (5 rats) 282 ± 38; 3♀, 2♂	With AD (7 rats) 355 ± 37; 3♀, 4♂
Superior cervical ganglia (left and right)	5·6 ± 1·9	71·6 ± 10·6	0·3 ± 0·1†	6·2 ± 1·8‡
Stellate ganglia (left and right)	19·1 ± 3·3	113·4 ± 11·5	3·5 ± 1·3§	17·6 ± 2·6‡
Thoracic chains (left and right)	73·9 ± 23·0	128·0 ± 18·0	11·9 ± 2·5†	31·1 ± 3·2‡
Retinas (left and right)	31·7 ± 8·5	53·9 ± 7·4	27·9 ± 6·7	51·2 ± 6·4
Pineal gland	1·54 ± 0·73 (4)‖	11·1 ± 2·2	0·68 ± 0·16 (3)	9·4 ± 2·1
Pituitary gland	94·1 (1)	152·0 ± 13·0 (6)	44·0 ± 36·0 (2)	146·0 ± 15·0 (6)

Results given as millimicromoles indoleacetic acid formed/hr/tissue ± S.E.

* Body weights, g ± standard error.
† $P < 0·05$, by t test in unpaired experiments.[16]
‡ $P < 0·001$.
§ $P < 0·01$.
‖ Number of animals.

cent of activity remained, as compared to control values. Antiserum treatment resulted in no significant changes in retinal, pineal, and hypophyseal monoamine oxidase activity, although the mean values (particularly those of the pineal glands) were decreased (Table 1). The monoamine oxidase activity of the spleen, submaxillary

* 1 N NaOH, 1 N HCl, followed by several washes with distilled water.

glands, kidneys, liver, lungs, atria, and uterus was reduced in immunosympathectomized rats, but only the activity of the spleen, submaxillary glands, kidneys, and liver showed a significant decrease (Table 2). The statistical analysis of these results pre-

TABLE 2. MONOAMINE OXIDASE ACTIVITY IN PERIPHERAL ORGANS OF CONTROL AND ANTISERUM-TREATED RATS IN THE PRESENCE AND ABSENCE OF EXOGENOUS ALDEHYDE DEHYDROGENASE (AD)

Tissue	Control rats		Antiserum-treated rats	
	No AD (5 rats)	With AD (7 rats)	No AD (5 rats)	With AD (7 rats)
	Micromoles indoleacetic acid formed/hr/tissue ± S.E.			
Spleen	1·81 ± 0·20	2·52 ± 0·35	1·09 ± 0·06*	2·05 ± 0·39†
Submaxillary glands	3·64 ± 0·45‡	4·91 ± 0·31	2·82 ± 0·46*	4·15 ± 0·43
Kidneys	4·29 ± 0·20	4·75 ± 0·22	3·15 ± 0·56	3·54 ± 0·27†
Liver	25·32 ± 1·51	29·30 ± 1·24	20·06 ± 2·97	23·49 ± 1·96*
Lungs	4·27 ± 0·23	5·34 ± 0·23	3·78 ± 0·58	4·97 ± 0·18
Left atrium	4·22 ± 0·87	12·81 ± 1·90	3·45 ± 0·84	11·74 ± 1·25
Right atrium	5·81 ± 0·94	13·36 ± 1·41	5·59 ± 1·28	13·13 ± 1·11
Uterus	1·48 ± 0·29§	4·11 ± 0·45§	1·36 ± 0·13§	3·86 ± 0·46§
	Micromoles indoleacetic acid formed/hr/g of protein ± S.E.			
Spleen	10·24 ± 1·60	13·29 ± 1·74	5·22 ± 0·49‖	9·51 ± 1·74‖
Submaxillary glands	24·18 ± 3·43‡	29·91 ± 2·35	18·50 ± 3·12*‡	22·51 ± 2·15
Kidneys	29·64 ± 2·40	29·80 ± 1·90	21·90 ± 4·50	21·00 ± 1·40†
Liver	141·40 ± 12·10	147·40 ± 8·12	117·90 ± 18·11	124·10 ± 7·02*
Lungs	31·30 ± 4·89	35·50 ± 4·19	26·00 ± 4·54	30·50 ± 1·77
Left atrium	29·50 ± 5·30	79·29 ± 9·81	22·96 ± 5·03	72·70 ± 9·80
Right atrium	37·72 ± 5·65	86·40 ± 12·34	36·50 ± 7·64	83·34 ± 9·19
Uterus	10·80 ± 1·88§	28·57 ± 4·58§	9·40 ± 1·08§	23·80 ± 2·51§

* $P < 0·05$, t test in paired experiments.[16]
† $P < 0·02$.
‡ Six animals.
§ Three animals.
‖ $P < 0·01$.

sented some difficulties. The decreases in monoamine oxidase activity observed in the spleen, submaxillary glands, kidneys, and liver were not uniformly significant in the four sets of results (per gram tissue and per gram protein in the presence and absence of exogenous aldehyde dehydrogenase). This apparent inconsistency is due to the small number of pairs in each set of results and to the relatively large variation in the differences between the pairs. The similarity of the variances within groups (in the absence and presence of exogenous aldehyde dehydrogenase), as indicated by the f test of ratios of variances, permitted the results to be combined for statistical analysis (Table 3).

Antiserum treatment had no effect on the monoamine oxidase activity of the ventricles, cardia, pyloric antrum, body of the stomach, and proximal and distal small intestine (Table 4).

As previously reported,[14] the addition of exogenous aldehyde dehydrogenase resulted in a faster rate of formation of the end product, indoleacetic acid, in a number of rat tissues. This was particularly marked in all neuronal tissues, ventricles, atria, and

TABLE 3. COMPARISON OF THE MEAN DIFFERENCES IN THE MONOAMINE OXIDASE ACTIVITY BETWEEN CONTROL AND ANTISERUM-TREATED RATS

Tissue	No. of pairs	Per g tissue		Per g protein	
		Mean difference ± S.E.	*P*	Mean difference ± S.E.	*P*
Spleen	10	0·723 ± 0·129	<0·001	4·15 ± 0·68	<0·001
Submaxillary glands	11	0·766 ± 0·328	<0·05	4·68 ± 1·49	<0·02
Kidneys	10	0·889 ± 0·266	<0·01	6·68 ± 1·64	<0·01
Liver	10	3·602 ± 1·466	<0·05	10·66 ± 4·46	<0·05

For the statistical analysis of data presented in Table 2, in the presence and absence of exogenous aldehyde dehydrogenase, results have been combined. *t* test was used in paired experiments.[16]

uterus. Near-saturating amounts of endogenous aldehyde dehydrogenase occurred in the stomach, liver, kidneys, and lungs. Tables 1–4 show that the actual results are not affected, whether or not saturating amounts of endogenous aldehyde dehydrogenase are present in a tissue; i.e. if a tissue from an antiserum-treated rat had a decreased activity in the absence of exogenous aldehyde dehydrogenase, it showed a similar decrease in the presence of this substance. This has been exemplified by the activities

TABLE 4. MONOAMINE OXIDASE ACTIVITY IN PERIPHERAL ORGANS OF CONTROL AND ANTISERUM-TREATED RATS IN THE PRESENCE AND ABSENCE OF EXOGENOUS ALDEHYDE DEHYDROGENASE (AD)

Tissue	Control rats		Antiserum-treated rats	
	No AD (5 rats)	With AD (7 rats)	No AD (5 rats)	With AD (7 rats)
	Micromoles indoleacetic acid formed/hr/tissue ± S.E.			
Ventricles	5·58 ± 0·31	18·41 ± 1·88	5·83 ± 0·34	18·00 ± 1·78
Cardia (stomach)	2·85 ± 0·19	3·51 ± 0·14	3·07 ± 0·08	3·42 ± 0·37
Pyloric antrum	4·64 ± 0·36	4·52 ± 1·42*	5·17 ± 0·14†	3·25‡
Body (stomach) + pyloric antrum	4·44 ± 0·36	4·54 ± 0·27	4·57 ± 0·50	4·90 ± 0·31
Small intestine (proximal)	3·84 ± 0·55	6·99 ± 0·42	5·08 ± 0·69	6·95 ± 0·69
Small intestine (distal)	6·02 ± 1·29	9·01 ± 0·82	6·90 ± 0·76	9·87 ± 0·68
	Micromoles indoleacetic acid formed/hr/g protein ± S.E.			
Ventricles	31·94 ± 4·40	85·04 ± 9·78	30·30 ± 2·97	83·55 ± 7·38
Cardia (stomach)	15·88 ± 2·44	17·67 ± 1·51	17·70 ± 1·68	16·30 ± 1·83
Pyloric antrum	33·20 ± 5·12	33·60 ± 13·50*	43·50 ± 11·18†	20·60‡
Body (stomach) + pyloric antrum	23·40 ± 2·68	25·10 ± 1·45	25·80 ± 2·50	28·90 ± 1·94
Small intestine (proximal)	26·31 ± 2·12	42·80 ± 4·98	31·20 ± 4·84	38·00 ± 2·88
Small intestine (distal)	39·08 ± 9·89	53·60 ± 2·41	45·74 ± 4·87	54·80 ± 2·41

* Two animals.
† Four animals.
‡ One animal.

seen in homogenates of the submaxillary glands (Table 2) and in the superior cervical ganglia (Table 1), the former containing nearly saturating amounts, and the latter containing rate-limiting amounts, of endogenous aldehyde dehydrogenase.

DISCUSSION

It is now accepted that treatment with antiserum of newborn rats (and probably mice) for 5 to 8 days does not result in complete regional or total immunosympathectomy. In our laboratory, treatment of newborn rats with the antiserum for 6 to 7 days destroyed approximately 88 per cent of the cell population of superior cervical ganglia and 81 per cent of stellate ganglia.[17] The inferences drawn from studies of the effect of antiserum treatment on some abdominal sympathetic ganglia are not satisfactory. Vogt[18] reported that celiac and mesenteric ganglia of rats (and probably other prevertebral ganglia) are not affected by postnatal antiserum treatment. The absence or near-absence of norepinephrine in the spleen of antiserum-treated rats[5] and mice treated prenatally and postnatally[19, 20] is strong evidence that one prevertebral ganglion at least (celiac), is affected by antiserum treatment. Zaimis *et al.*[21] reported that the celiac ganglion is affected, but that the superior mesenteric ganglion is not. Unpublished observations from this laboratory indicate that, in mice, the ganglionic complex in close apposition to the celiac and superior mesenteric arteries is affected by antiserum treatment.[20]

In the present study, small and consistent but not always statistically significant decreases in monoamine oxidase activity were noted in several organs and tissues of immunosympathectomized rats. In a previous study of dopa-decarboxylase activity in tissues from antiserum-treated rats,[5] a similar observation was noted; i.e. in some tissues there were usually small but significant decreases (atria, spleen, submaxillary glands, uterus, and sympathetic ganglia), while in other tissues no significant decreases were noted (kidneys, intestine, lungs, and ventricles). This can be explained by the fact that methods at present available for measuring dopa-decarboxylase and monoamine oxidase activities *in vitro* do not distinguish decarboxylases and monoamine oxidase present in peripheral nonadrenergic sites from the decarboxylase and monoamine oxidase associated with sympathetic structures and catecholamine metabolism. If, in a tissue, the proportion of the non-neuronal enzyme to the neuronal enzyme is large, it may be impossible to show significant decreases in enzyme activity after sympathetic denervation or after antiserum treatment. This is further complicated by the rather large variations in the activity of these enzymes in a particular tissue from rat to rat, which make the interpretation of the small changes, encountered between control and antiserum-treated rats, very difficult.

The above study does show that in some tissues (e.g. spleen and submaxillary glands) a relation exists between the sympathetic innervation and monoamine oxidase activity. The superior cervical ganglion lends itself to exemplification of the relation between various parameters which are essential to the functioning of the sympathetic nervous system. In the present and previous investigations,[5, 17] it has been shown that in superior cervical ganglia from antiserum-treated rats, the monoamine oxidase activity decreased to 8 per cent, dopa-decarboxylase activity to 5 per cent, norepinephrine content to 8 and 12 per cent (two studies), cell counts to 12 per cent, and the action potential *in vitro* to 13 per cent, of control values.

The incomplete destruction of the sympathetic nervous system by the antiserum may be responsible for the rather small changes in monoamine oxidase activity described here. However, surgical denervation,[7–12] particularly ganglionectomy which one could assume would result in more complete regional denervation, does not cause more striking decreases in monoamine oxidase activity either, except in the cat's nictitating membrane,[7] and in one study with rat pineal glands.[11] The decrease in monoamine oxidase activity of denervated rat submaxillary glands is similar to the decrease in monoamine oxidase activity of submaxillary glands from antiserum-treated rats of the present study. Thus, Snyder *et al.*[11] reported a decrease of 28 per cent of monoamine oxidase activity in denervated submaxillary glands as compared to to the innervated side, while immunosympathectomy resulted in a 22·5 per cent decrease in enzyme activity of the submaxillary glands.

The large decreases (58, 60, and 71 per cent) in monoamine oxidase activity of the heart, spleen, and submaxillary gland, respectively, after antiserum treatment, as previously reported by Levi-Montalcini and Angeletti,[6] cannot be explained readily when compared to the smaller decreases in the spleen and submaxillary glands and the absence of a decrease in ventricles and atria in this investigation. Different assays were used in these two studies. Levi-Montalcini and Angeletti measured monoamine oxidase activity in crude homogenates by the oxygen uptake at 37° in Warburg manometers for 60 min, using tyramine as the substrate. In the present experiments the end product of the reaction, indoleacetic acid, was measured with tryptamine as the substrate. Except for neuronal homogenates in the absence of exogenous aldehyde dehydrogenase, incubation periods were 20 min or less (dependent upon the tissue). The methodology has been studied in detail in this laboratory,[14] and the assay of Lovenberg *et al.*[13] was found suitable for quantitative, reproducible comparison of monoamine oxidase activity in crude homogenates from rat tissues. In each assay, the third aliquot (see Methods) was used either for the measurement of a second time period (to assure linearity) or for the addition of the monoamine oxidase inhibitor, β-phenylisopropylhydrazine (to assure specificity).

It is unfortunate that this report does not aid in clarifying the results of Snyder *et al.*[11] and Håkanson and Owman[12] on the monoamine oxidase activity in the denervated pineal gland of the rat. In the present study, the mean value of the pineal monoamine oxidase activity after antiserum treatment is decreased, but not to a statistically significant extent.

The disturbing result presented here is the decrease in hepatic monoamine oxidase activity. If judged by the relatively low norepinephrine content of rat liver,[5] sympathetic nerve endings are less numerous in this organ than, for instance, in spleen and submaxillary glands. It is difficult to reconcile the decrease in hepatic monoamine oxidase activity with the postulate that small changes will not be detected readily when the proportion of the neuronal monoamine oxidase to the non-neuronal monoamine oxidase activity is small.

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