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MONOAMINE OXIDASE ACTIVITY OF THE CAT NICTITATING MEMBRANE AND SUPERIOR CERVICAL GANGLION UNDER VARIOUS EXPERIMENTAL CONDITIONS*†

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Abstract—Monoamine oxidase activity of the cat nictitating membrane and superior cervical ganglion was studied using a fluorimetric technique after chronic decentralization and denervation and pretreatment with reserpine and pheniprazine. Catecholamine content of the cat nictitating membrane and superior cervical ganglion was measured by using a fluorimetric technique after reserpine and pheniprazine pretreatments. Monoamine oxidase activity of the nictitating membrane was reduced in all procedures studied, while enzyme activity of the superior cervical ganglion was unchanged by sectioning of the preganglionic fiber (decentralization). Catecholamine content of the nictitating membrane was unchanged by pheniprazine treatment, while that of the superior cervical ganglion increased. Results are discussed in terms of the possible differences in the turnover rates of norepinephrine in the nictitating membrane and superior cervical ganglion. The effect of monoamine oxidase inhibition on reserpine-induced depletion of catecholamines in the cat nictitating membrane and in rat heart was also studied. The catecholamine content of the cat nictitating membrane 18–24 hr after treatment with reserpine or with pheniprazine plus reserpine was reduced to the same extent. Similar results were obtained with the rat heart. The data support the concept that monoamine oxidase inhibition does not prevent the reserpine-induced release of catecholamines.

IN A PREVIOUS study from this laboratory, it was reported that the catecholamine content of the cat denervated superior cervical ganglion increased while the catecholamine content of the ipsilateral decentralized§ nictitating membrane remained unchanged after sectioning of the preganglionic fiber to the superior cervical ganglion.[1] Similar observations were made in the rat superior cervical ganglion and salivary

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§ For convenience in this paper the term "decentralization" will refer to sectioning of the preganglionic fibers to the superior cervical ganglion, bearing in mind that this procedure accomplishes denervation of the superior cervical ganglion (removal of its ultimate nerve supply) and decentralization of the nictitating membrane (removal of its penultimate nerve supply). The term "denervation" will refer to sectioning of the postganglionic fibers of the superior cervical ganglion or extirpation of the superior cervical ganglion and part of the postganglionic nerves. This accomplishes denervation of the nictitating membrane (removal of its ultimate nerve supply). The terms "nictitating membrane" and "membrane" will refer to the smooth muscles of the nictitating membrane. In all cases concentrations of catecholamines and monoamine oxidase activities refer to the concentrations and activities in the smooth muscle of the membrane structure.

gland by Fisher and Snyder.[2] Pretreatment with monoamine oxidase (MAO) inhibitors increases the levels of endogenous catecholamines in the cat superior cervical ganglion,[3] but not in the cat nictitating membrane (see Results). In view of the findings that sectioning of the preganglionic fiber to the superior cervical ganglion and pretreatment with MAO inhibitors produce highly significant changes in the catecholamine content of the superior cervical ganglion but not in the nictitating membrane, it was of interest to determine whether there was any relationship between the catecholamine content and MAO activity of these tissues. Estimation of the MAO activity of the nictitating membrane and superior cervical ganglion was carried out after denervation, decentralization, reserpine pretreatment and pheniprazine pretreatment.

Axelrod *et al.*[4] reported that the MAO inhibitors prevent reserpine depletion of tritium-labeled norepinephrine in rat heart. Spector *et al.*[5] and Costa *et al.*[6] proposed that the MAO inhibitors did not prevent the release of norepinephrine from its binding sites by reserpine but prevented the rapid decline in amines by inhibiting their metabolism. The present study describes the effect of pheniprazine treatment on the catecholamine content of the cat superior cervical ganglion and nictitating membrane and the effect of pheniprazine on reserpine depletion of catecholamines in cat nictitating membrane and superior cervical ganglion and in rat heart.

METHODS

Monoamine oxidase assay. Fresh ganglia and smooth muscles of the nictitating membranes were homogenized in 1·0 ml of cold 0·25 M sucrose solution. The average weights of the tissues employed were: 11·19 $\pm$ 0·29 mg for superior cervical ganglia, 13·02 $\pm$ 0·51 mg for stellate ganglia, and 21·88 $\pm$ 2·52 mg for nictitating membranes. An 0·8-ml aliquot of the homogenate was employed for estimation of enzyme activity. MAO activity was estimated according to the method described by Lovenberg *et al.*,[7] using exogenous aldehyde dehydrogenase in the incubation mixture for ganglia and membranes. A 30-min incubation period was employed for both membranes and ganglia. The reaction for both tissues was linear over a 60-min incubation period and this was checked periodically throughout the study. The MAO activity is expressed as micromoles of indoleacetic acid formed per gram of tissue per hour of incubation (μmoles IAA/g/hr).

Catecholamine assay. The catecholamine content of the cat nictitating membrane and superior cervical ganglion and the rat heart was estimated according to the method described by Kirpekar *et al.*[1]

Treatment of animals. Surgical procedures were carried out under aseptic conditions on adult cats of either sex anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg). For denervation, usually the right superior cervical ganglion was removed with a small amount of post-ganglionic fiber and the cats were allowed to recover. For decentralization, the preganglionic fiber (1–2 cm) of one superior cervical ganglion (usually the right) was removed and the cats were allowed to recover. At the appropriate day after surgery, the animals were anesthetized and the nictitating membranes on the operated side as well as on the contralateral normal side were dissected according to the method described by Cervoni *et al.*[8] The superior cervical and stellate ganglia were also removed for analysis.

Reserpine (Serpasil, Ciba) was administered *in vivo* 18–24 hr prior to removal of tissues for analysis. Cats received 5 mg per kg i.p. and rats received 10 mg per kg i.p.

β-Phenyl-*iso*propyl hydrazine (pheniprazine, PIH; Catron, Lakeside) was administered daily i.p. at a dose of 3 mg per kg per day for 7 days to cats and rats prior to the removal of tissues for analysis. Pheniprazine-pretreated cats received only 1 mg per kg i.p. of reserpine.

RESULTS

Monoamine oxidase activity of the cat superior cervical ganglion. MAO activity of superior cervical ganglia obtained from normal, untreated and unoperated cats was 3·86 ± 0·15 μmoles IAA/g/hr. The MAO activity of ganglia obtained from cats pretreated with reserpine was not significantly different from that of normal ganglia ($P = 0·11$). Pretreatment with β-phenyl-isopropyl hydrazine (PIH) produced about an 80 per cent reduction in MAO activity ($P < 0·001$). Denervation of the superior ganglion (12–16 days) produced no change in MAO activity. The MAO activity of the contralateral normal ganglia in this series was 4·38 ± 0·44 μmoles IAA/g/hr, while the MAO activity of the denervated ganglia was 4·38 ± 0·55 ($P > 0·9$). These results are summarized in Fig. 1.

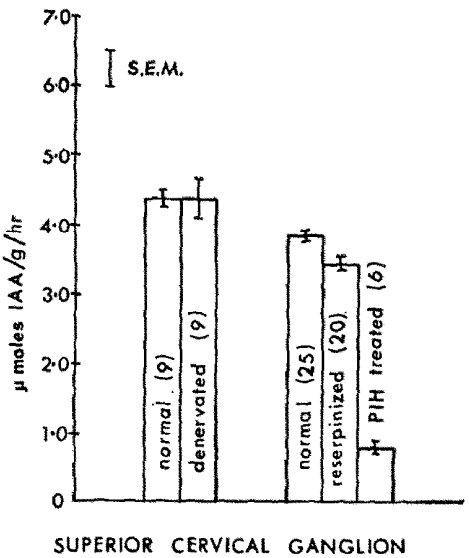

FIG. 1. Monoamine oxidase activity in superior cervical ganglion of normal, reserpine-pretreated and pheniprazine-pretreated (PIH) cats and after chronic sectioning of the preganglionic fiber to the superior cervical ganglion (denervation). Numbers in parentheses indicate number of experiments. Vertical lines are the standard errors of the means.

Monoamine oxidase activity of the cat stellate ganglion. The MAO activity of normal cat stellate ganglion was 3·85 ± 0·34 μmoles IAA/g/hr. The results obtained on the MAO activity of normal stellate and superior cervical ganglia are in agreement with the data published by Lovenberg *et al.*[7] on cat ganglia. Reserpine pretreatment produced about a 20 per cent reduction in mean MAO activity. The difference was of borderline significance from the MAO activity of normal ganglia ($P = 0·09$). Pretreatment with PIH reduced MAO activity about 75 per cent ($P < 0·001$). These results are summarized in the bar graph in Fig. 2.

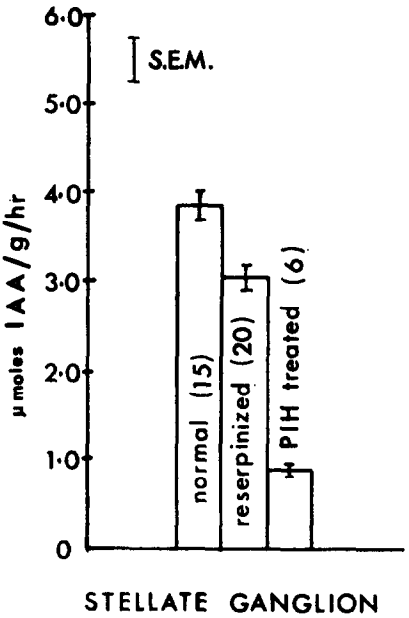

FIG. 2. Monoamine oxidase activity in cat stellate ganglion of normal, reserpine-pretreated and pheniprazine-pretreated cats. Numbers in parentheses represent number of experiments. Vertical lines represent the standard errors of the means.

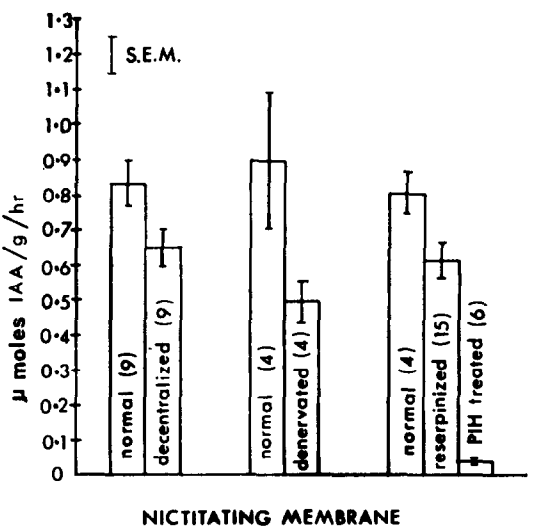

FIG. 3. Monoamine oxidase activity in nictitating membrane of normal, reserpine-pretreated and pheniprazine-pretreated cats and after chronic sectioning of the preganglionic fiber to the superior cervical ganglion (decentralization) and chronic extirpation of the superior cervical ganglion (denervation). Numbers in parentheses represent the number of experiments. Vertical lines indicate the standard errors of the means.

Monoamine oxidase activity of the cat nictitating membrane. The bar graph in Fig. 3 summarizes the data on the effects of denervation, decentralization, reserpine pretreatment and PIH pretreatment on the cat nictitating membrane. The MAO activity of the membrane was considerably less than that of ganglion. The mean MAO activity in seventeen normal membranes was 0·86 ± 0·06 μmoles IAA/g/hr. In membranes which had been decentralized 12–16 days, there was about a 20 per cent decrease in mean MAO activity ($P = 0·04$). Membranes which had been denervated for 7–15 days exhibited a 50 per cent reduction in MAO activity (0·93 ± 0·17 for normal and 0·47 ± 0·02 μmoles IAA/g/hr for denervated nictitating membrane; $P < 0·05$). In two experiments in which denervation was carried out to 44 and 47 days, the MAO activity of the denervated membranes appeared to have returned to normal levels The MAO activities of the denervated membranes at 44 and 47 days were 0·81 and 0·67 μmoles IAA/g/hr, respectively, while the corresponding contralateral control membranes had MAO activities of 0·89 and 0·78 μmoles IAA/g/hr. These findings are in agreement with those of Burn and Robinson[9] on the MAO activity in normal and denervated cat nictitating membrane. Reserpine pretreatment reduced MAO activity of the nictitating membrane 25 per cent ($P = 0·01$), while PIH pretreatment reduced MAO activity of the membrane 95 per cent ($P < 0·001$).

Effect of pheniprazine and reserpine on the catecholamine content of the cat superior cervical ganglion. Table 1 shows the effect of PIH and reserpine on the catecholamine

TABLE 1. CATECHOLAMINE CONTENT OF THE CAT SUPERIOR CERVICAL GANGLION AND NICTITATING MEMBRANE AFTER PRETREATMENT WITH RESERPINE AND PHENIPRAZINE, ALONE AND IN COMBINATION

Treatment	Mean concn (μg/g fresh superior cervical ganglion)*		Mean concn (μg/g fresh nictitating membrane)*	
	NE	E	NE	E
None†	6·86 ± 0·31 (16)	0·19 ± 0·05 (16)	3·90 ± 0·34 (5)	0·05 ± 0·03 (5)
PIH‡	14·51 ± 0·67 (17)	0·65 ± 0·01 (17)	3·15 ± 0·79 (4)	0·15 ± 0·03 (4)
PIH + reserpine§	0·51 ± 0·20 (6)	0·42 ± 0·05 (6)	undetectable (6)	0·07 ± 0·05 (6)
Reserpine†, ‖	0·28 ± 0·08 (4)	not determined	0·05 ± 0·02 (12)	0·03 ± 0·01 (12)

* Concentrations are expressed as the free bases ± S.E. of the mean; number of experiments in parentheses.

† Obtained from the data of Kirpekar *et al.*[1]

‡ PIH (3 mg/kg) was administered i.p. daily for 7 days.

§ PIH (3 mg/kg) was administered i.p. daily for 7 days, then on the seventh day reserpine (1 mg/kg, i.p.) was administered. The tissues were removed 18–24 hr after reserpine administration.

‖ Reserpine (1 mg/kg, i.p.) was administered 18–24 hr prior to removal of tissues for analysis.

content of the cat superior cervical ganglion. PIH treatment produced a 2- to 3-fold increase in norepinephrine (NE) and epinephrine (E) concentrations ($P < 0·001$ in both instances). Reserpine reduced the NE content of normal and PIH-treated ganglia about 96 per cent ($P < 0·001$ in both groups). There was no significant difference in the extent of NE depletion between the normal or PIH-pretreated groups by reserpine ($P > 0·3$). On the other hand, reserpine produced only a 33 per cent depletion of E in the PIH-treated group. Since the effect of reserpine on the E content of normal ganglia was not determined, comparison of the extent of E depletion by reserpine in normal and PIH-treated groups could not be carried out.

Effect of pheniprazine and reserpine on the catecholamine content of the cat nictitating membrane. PIH produced no change in the NE or E concentrations in the nictitating membrane of the cat (see Table 1; $P > 0{\cdot}4$ for NE and $P > 0{\cdot}05$ for E). Reserpine produced a marked depletion of NE from the normal membranes, and a depletion from the PIH-pretreated membranes to undetectable levels ($P < 0{\cdot}001$ in both groups). Reserpine had no effect on the E content of normal and PIH-treated tissues ($P < 0{\cdot}1$ in both groups).

Effect of pheniprazine and reserpine on the catecholamine content of the rat heart. In the rat heart, PIH produced about a 40 per cent increase in mean NE content, which was of borderline significance ($P \sim 0{\cdot}09$); E, on the other hand, was significantly increased over normal ($P < 0{\cdot}01$). The data are summarized in Table 2. Similar increases in total catecholamines after treatment with MAO inhibitors were reported by Muscholl[10] and Crout *et al.*[11] in rat heart, by Pletscher[12] in guinea-pig heart, and by Sanan and Vogt[3] in rabbit atrium. In PIH-pretreated rats, reserpine treatment reduced the levels of both E ($P < 0{\cdot}01$) and NE ($P = 0{\cdot}03$) in the heart.

TABLE 2. CATECHOLAMINE CONTENT OF THE RAT HEART AFTER TREATMENT WITH PHENIPRAZINE ALONE AND IN COMBINATION WITH RESERPINE

Treatment	N	Mean NE concn (μg/g fresh tissue)*	Mean E concn (μg/g fresh tissue)*
None	4	$0{\cdot}82 \pm 0{\cdot}14$	$0{\cdot}04 \pm 0{\cdot}02$
PIH†	12	$1{\cdot}15 \pm 0{\cdot}09$	$0{\cdot}12 \pm 0{\cdot}03$
PIH + reserpine‡	4	undetectable	$0{\cdot}04 \pm 0{\cdot}01$

* Concentrations are expressed as the free bases ± S.E. of the mean.
† PIH (3 mg/kg) was administered i.p. daily for 7 days.
‡ PIH (3 mg/kg) was administered i.p. daily for 7 days, then on the seventh day reserpine (10 mg/kg) was administered. The hearts were removed 18–24 hr after reserpine administration.

DISCUSSION

After chronic sectioning of the preganglionic fiber to the superior cervical ganglion (decentralization), the catecholamine content of the ganglion increases, while the catecholamine content of the effector organ does not change (cat nictitating membrane;[1] rat salivary gland[2]). In the present study, pretreatment with the MAO inhibitor, pheniprazine, increased the catecholamine content of the cat superior cervical ganglion almost 2-fold, while the catecholamine content of the nictitating membrane was not altered. No correlation was found between catecholamine levels and MAO activity in the cat superior cervical ganglion and nictitating membrane after decentralization and pheniprazine treatment, since decentralization only affected the enzyme activity of the membrane while pheniprazine affected that of the ganglion and of the membrane. Fisher and Snyder[2] showed that, after decentralization, the norepinephrine turnover rate was reduced to a greater extent in the superior cervical ganglion than in the salivary gland of the rat. This would account for the increase in the catecholamine content of the ganglion but not in the effector organ. In the present study pheniprazine treatment produced changes in ganglionic and membrane norepinephrine similar to those demonstrated after decentralization.[1, 2] It is conceivable that the MAO inhibitors also decrease the norepinephrine turnover rate in the superior cervical ganglion to a

greater extent than in the nictitating membrane. Recently, Neff and Costa[13] showed that MAO inhibition reduced norepinephrine turnover rate in rat heart.

Reserpine pretreatment reduced the MAO activity of the nictitating membrane about 20 per cent, as did decentralization. Izumi *et al.*[14] reported that MAO activity in guinea-pig heart slices and homogenates was stimulated 2·5 hr after reserpine treatment *in vitro*. The author is unaware of any studies reporting a reduction of MAO activity *in vivo* in other adrenergic neuroeffector systems. Whether a 20 per cent reduction in MAO activity is of any physiological or pharmacological significance is difficult to assess. MAO inhibitors that do not inhibit the uptake of catecholamines, e.g. iproniazid, do not potentiate norepinephrine-induced contraction of smooth muscle[15–20] or alter the sensitivity of the heart to norepinephrine.[21] The reduction in enzyme activity after decentralization or reserpine treatment may be due to the lack of "available transmitter", which may be required to maintain "normal enzyme activity".

From the literature, it is evident that there is a difference in the level of catecholamines from tissues obtained from normal animals and those pretreated with an MAO inhibitor if measurements are made 1–6 hr after reserpine treatment.[4–6] However, measurements made at longer time intervals after reserpine treatment (18–24 hr), as in the present study, indicate that normal and MAO-inhibited groups are depleted 95–98 per cent. The data support the concept suggested by Spector *et al.*[5] and Costa *et al.*[6] that the MAO inhibitors slow the reserpine-induced release of norepinephrine but do not prevent the release of norepinephrine as suggested by Axelrod *et al.*[4]

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REFERENCES

1. S. M. Kirpekar, P. Cervoni and R. F. Furchgott, *J. Pharmac. exp. Ther.* **135**, 180 (1962).
2. J. E. Fisher and S. Snyder, *J. Pharmac. exp. Ther.* **150**, 190 (1965).
3. S. Sanan and M. Vogt, *Br. J. Pharmac. Chemother.* **18**, 109 (1962).
4. J. Axelrod, G. Hertting and R. W. Patrick, *J. Pharmac. exp. Ther.* **134**, 325 (1961).
5. S. Spector, R. Kuntzman, P. A. Shore and B. B. Brodie, *J. Pharmac. exp. Ther.* **130**, 256 (1960).
6. E. Costa, A. M. Revzin, R. Kuntzman, S. Spector and B. B. Brodie, *Science, N.Y.* **133**, 1822 (1961).
7. W. Lovenberg, R. J. Levine and A. Sjoerdsma, *J. Pharmac. exp. Ther.* **135**, 7 (1962).
8. P. Cervoni, T. C. West and L. D. Fink, *J. Pharmac. exp. Ther.* **116**, 90 (1956).
9. J. H. Burn and J. Robinson, *Br. J. Pharmac. Chemother.* **7**, 304 (1952).
10. E. Muscholl, *Experientia* **15**, 428 (1959).
11. J. R. Crout, C. R. Creveling and S. Udenfriend, *J. Pharmac. exp. Ther.* **132**, 269 (1961).
12. A. Pletscher, *Experientia* **14**, 73 (1958).
13. N. H. Neff and E. Costa, *Life Sci.* **5**, 951 (1966).
14. F. Izumi, M. Oka, H. Yoshida and A. Imaizumi, *Life Sci.* **6**, 2333 (1967).
15. E. C. Griesemer, J. Barsky, C. A. Dragstedt, J. A. Wells and E. A. Zeller, *Proc. Soc. exp. Biol. Med.* **84**, 699 (1953).
16. J. H. Burn, F. J. Philpot and U. Trendelenburg, *Br. J. Pharmac. Chemother.* **9**, 423 (1954).
17. R. F. Furchgott, *Pharmac. Rev.* **7**, 183 (1955).
18. H. Schmitt and P. Gonnard, *Arch. int. Pharmacodyn. Thér.* **108**, 74 (1956).
19. K. Kamijo, G. B. Koelle and H. H. Wagner, *J. Pharmac. exp. Ther.* **117**, 213 (1956).
20. T. H. Tsai and W. W. Fleming, *J. Pharmac. exp. Ther.* **148**, 40 (1965).
21. C. B. Smith, *J. Pharmac. exp. Ther.* **151**, 207 (1966).