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The influence of adrenalectomy on monoamine oxidase and NADH cytochrome c reductase in the rat heart

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The effect of adrenalectomy on the activities of monoamine oxidase (MAO), NADH cytochrome c reductase (NCR), succinate dehydrogenase, malate dehydrogenase, fumarase, NAD^+ nucleosidase and acid phosphatase in homogenates of rat hearts was examined. Besides MAO only the NCR activity increased. However, both the total and the rotenone-insensitive NCR activities increased, with that of the rotenone-insensitive being about half of the total, which indicated that the effect of adrenalectomy was exerted on components of this enzyme localized on both the inner and outer membranes of the mitochondrion. The lack of effect on the other enzymes suggests that adrenalectomy has a relatively selective action on MAO and NCR, and does not work by a generalized increase in protein synthesis or by an effect on the FAD cofactor. The MAO increase was seen with a variety of substrates, and was due to a rise in Vmax without change in Km. The response to adrenalectomy in the summer differed from that seen in the winter. The possible reasons for these effects of adrenalectomy are discussed.

It is now well established that in the rat, adrenalectomy leads to an increase in the specific activity of monoamine oxidase (MAO; EC 1.4.3.4) in some tissues. This effect is most clearly seen in the rat heart (Avakian & Callingham, 1968; Bhagat, 1969; Westfall & Osada, 1969; Caesar, Collins & Sandler, 1970; Holzbauer & Youdim, 1972; Sampath, Shih & Clarke, 1972; Parvez & Parvez, 1973). Treatment of the adrenalectomized animals with hydrocortisone has been shown to prevent the increase in MAO activity in the heart (Avakian & Callingham, 1968; Parvez & Parvez, 1973) and in the brain and liver (Parvez & Parvez, 1973), while Sampath & Clarke (1972) found that treatment with dexamethasone was effective in preventing the increase in MAO activity in the vas deferens. The mechanism responsible for this relation between circulating glucocorticoid concentrations and MAO activity is unknown, although it has been suggested that the increase in MAO activity that follows adrenalectomy in the rat is due to a general increase in protein synthesis in the mitochondria (Parvez & Parvez, 1973).

MAO is a flavin-containing enzyme located in the outer membrane of the mitochondrion (see Tipton, 1975). Another flavoprotein is also known to be associated with the outer membrane. This comprises part of the activity of NADH cytochrome c reductase (NCR; EC 1.6.99.3). The remaining activity is found in the mitochondrial inner membrane and in the microsomes (see Ernster & Kuylenstierna, 1969). The inner membrane fraction differs from the other two, since it is sensitive to inhibitors of electron transport such as antimycin A and rotenone. Although no functional connexion between MAO and NCR has been suggested, both enzyme activities in rat liver mitochondria are reduced by treatment of the animals with (−)-thyroxine for several days (Okamoto, 1971). Preliminary experiments (Callingham & Della Corte, 1971) showed that the NCR activity in homogenates and mitochondrial fractions from rat hearts increased following adrenalectomy of the animal. However, in neither study was an attempt made to distinguish between effects on rotenone-sensitive and insensitive components of the NCR activity. In the rat heart, adrenalectomy did not affect succinate dehydrogenase from the inner mitochondrial membrane or malate dehydrogenase and fumarase from the matrix.

In the present experiments, the rotenone-insensitive and total NCR activity together with MAO and other marker enzymes have been examined in an attempt to determine whether or not the effect of adrenalectomy in the rat is localized to those enzymes in the outer membrane of the mitochondria in the heart. Several substrates for MAO have also been used to see if any change is produced in the nature of the enzyme or in its kinetic parameters due to the fall in circulating corticosteroids.

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MATERIALS AND METHODS

Materials

The radioactive substrates for MAO, [^{3}H]tyramine and [^{14}C]β-phenethylamine, were obtained from New England Nuclear, GMBH, Dreieichenhain, Germany; [^{3}H]5-hydroxytryptamine and [^{3}H]dopamine, from the Radiochemical Centre, Amersham, U.K.; and [^{14}C]benzylamine from ICN Pharmaceuticals, Hersham, U.K. Clorgyline hydrochloride was a gift from May & Baker, Dagenham, Ltd., U.K. All other reagents were of analytical grade where possible.

Male and female rats were obtained from A. J. Tuck & Son, Rayleigh.

Methods

Adrenalectomy. Rats were adrenalectomized through a dorsal incision under ether anaesthesia. After operation the animals were maintained on a normal diet with free access to 0·9% NaCl solution. Sham operations were performed to provide appropriate age-matched controls.

Preparation of heart homogenates. Adrenalectomized and control animals were killed by a blow on the head or by cervical dislocation and their hearts rapidly removed.

Crude homogenates were prepared by a modification of the method of Ernster & Nordenbrand (1967) as described earlier (Della Corte & Callingham, 1977). Briefly, the hearts were homogenized first with a loose-fitting Dounce homogenizer followed by a conical all-glass homogenizer. The medium was modified from that described by Chappell & Perry (1954) by the omission of $MgCl_2$ and the replacement of EDTA by EGTA. The homogenates were diluted to a final concentration of 10% w/v, and centrifuged at 650 g for 10 min twice to remove nuclei and cell debris.

Enzyme assays. MAO was assayed by the method of McCaman, McCaman & others (1965) as modified by Callingham & Laverty (1973), using radioactively labelled tyramine, benzylamine, β-phenethylamine, dopamine and 5-HT as substrates. In experiments with clorgyline, the homogenates were pre-incubated with the inhibitor for 20 min before the addition of substrate. Specific activites were expressed in (nmol substrate metabolized) (mg protein)$^{-1}$h^{-1}.

NADH cytochrome c reductase (NCR) was measured by a modification of the method of Nason & Vasington (1963). The rate of oxidation of NADH was measured by following the decrease in fluorescence at an emission wavelength of 455 nm (excitation, 355 nm) in 250 μl of a reaction mixture containing 80 mM potassium phosphate buffer, pH 7·5, horse heart ferricytochrome c 1·6 mg ml^{-1} in water, 4 mM KCN, 0·048 mM NADH and 5 to 20 μl of tissue homogenate. Specific activity was expressed in (nmol NADH oxidized) (mg protein)$^{-1}$min^{-1}.

Succinate dehydrogenase (SDH) was measured by following the reduction of 2,6-dichlorophenolindophenol in the presence of succinate by the method of Green, Mii & Kohout (1955). Specific activity was expressed in (nmol succinate oxidized) (mg protein)$^{-1}$ min^{-1}.

Malate dehydrogenase (MDH) was measured by following the reduction of NAD^+ at 300 nm by the method of Siegel & Englard (1961). Specific activity was expressed in (nmol NAD^+ reduced) (mg protein)$^{-1}$min^{-1}.

Fumarase was measured by following the increase in absorption at 250 nm produced by the formation of fumarate from L-malate using the method of Racker (1950). Specific activity was expressed in (nmol fumarate formed) (mg protein)$^{-1}$min^{-1}.

Acid phosphatase was measured with *p*-nitrophenylphosphate as substrate, as described by Linhard & Walter (1963). Specific activity was expressed in (nmol *p*-nitrophenol formed) (mg protein)$^{-1}$min^{-1}.

NAD^+ nucleosidase (NADase) was measured by the method of Zatman, Kaplan & Colowick (1953), based on the cyanide reaction of NAD^+. Specific activity was expressed in (nmol NAD^+-cyanide complex formed) (mg protein)$^{-1}$min^{-1}.

Protein was measured by the micro biuret method of Goa (1953), with bovine serum albumin as standard.

Kinetic measurements of MAO activity. The substrates for MAO were used over a range of final concentrations from 0·1 to 1·0 mM. At each substrate concentration, 4 to 6 different incubation times were used. Each incubation was performed in duplicate. Initial velocities were calculated from progress curves both graphically and by computer program. The Km and Vmax values were calculated by the method of Wilkinson (1961). Statistical significance between groups was calculated by Student's *t*-test on the absolute values of the specific activities, or by the confidence limits of a ratio (Goldstein, 1967).

RESULTS

Effect of adrenalectomy on the specific activities of MAO, NCR and other heart enzymes

Rats were killed between 15 and 60 days after adrenalectomy together with their appropriate age-matched controls. The specific activities of MAO, rotenone-insensitive and total NCR, SDH, MDH,

fumarase, acid phosphatase and NADase were measured in homogenates of the hearts from each group.

It was found that the specific activity of the NCR increased with the age of the rat, in a manner that resembled the age-related increase in MAO specific activity (see Table 1 and Della Corte & Callingham, 1977). Since an analysis of the kinetics of the effects of adrenalectomy is not the present purpose, no mathematical device has been used to compensate for the increase in activity of either MAO or NCR as the animals grew older. No such age-related increase in specific activity was seen with the other enzymes examined.

Table 1. *Specific activities of MAO, total and rotenone-insensitive NCR in homogenates of normal rat hearts.*

Approx. age (days)	Body wt (g)	Heart wt (mg)	Enzyme activities in units MAO	NCR-T	NCR-RI
35	119 ± 2	404 ± 7	53·0 ± 3	39·7 ± 7	21·5 ± 3
38	127 ± 1	441 ± 2	52·4 ± 13	36·2 ± 4	—
42	150 ± 3	466 ± 10	82·7 ± 6	31·2 ± 2	—
45	166 ± 1	493 ± 5	125 ± 18	40·9 ± 2	—
49	186 ± 4	523 ± 20	108 ± 20	38·4 ± 2	—
54	215 ± 9	613 ± 31	154 ± 9	47·1 ± 2	—
58	232 ± 5	649 ± 34	298 ± 25	50·2 ± 3	21·8 ± 1
90	313 ± 8	823 ± 31	299 ± 36	76·7 ± 14	34·6*

Values are means ± s.e.m. of groups of 3–6 animals obtained from two separate batches. * only two values. MAO specific activity is expressed in: (nmol tyramine metabolized) (mg protein)$^{-1}$h^{-1}, total (T) and rotenone-insensitive (RI) NCR activities are expressed in: (nmol NADH oxidized) (mg protein)$^{-1}$ min^{-1}.

The control activities of the marker enzymes (in units ± s.e.m.) were found to be: SDH; 13·42 ± 1·42, MDH; 1925 ± 278, fumarase; 2157 ± 55, acid phosphatase; 7·12 ± 1·22 and NADase; 5·82 ± 1·22, for 4 to 9 homogenates.

Fig. 1 summarizes the relative changes in enzyme activities following adrenalectomy. The only enzyme besides MAO that increased in activity was the NCR. Both the rotenone-insensitive, located in the outer membrane of the mitochondrion and in the microsomes, and the total NCR activity, which also comprises the NCR on the mitochondrial inner membrane, were increased by the same proportion. The same proportional increase in total NCR activity was found in four experiments where washed mitochondrial fractions were used instead of crude homogenates.

No change was seen in the activities of the inner mitochondrial membrane marker, SDH or in MDH or fumarase from the matrix. Lysosomal acid phosphatase and microsomal NADase were also unaffected.

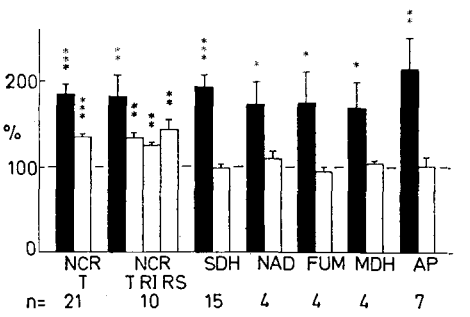

FIG. 1. The effect of adrenalectomy on MAO activity (% of control), (closed columns); NADH cytochrome c reductase, (NCR); total activity, (T); rotenone-sensitive, (RS); rotenone-insensitive, (RI); succinate dehydrogenase, (SDH); NAD$^+$ nucleosidase, (NAD); fumarase, (FUM); malate dehydrogenase, (MDH) and acid phosphatase, (AP) activities in homogenates of rat hearts. Specific activities of the enzymes are expressed as percentages of their corresponding controls (dashed line) as means ± s.e. of a ratio: n = number of observations. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; when compared with control, all other differences are not significant.

Effect of adrenalectomy on heart MAO measured with several substrates

The activity of the heart MAO from normal and adrenalectomized rats was measured with tyramine, β-phenethylamine, dopamine, 5-HT and benzylamine as substrates. Fig. 2 summarizes the results obtained from 4 groups of adult rats of different ages, and killed at various times after adrenalectomy.

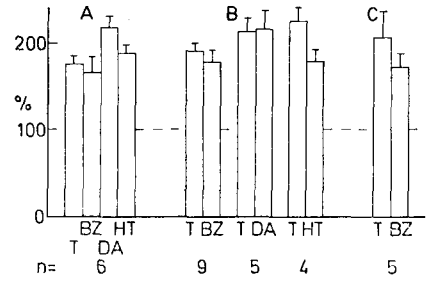

FIG. 2. The effect of adrenalectomy on the metabolism of tyramine, (T); benzylamine, (BZ); dopamine, (DA) and 5-HT, (HT). Specific activity of MAO with the various substrates is expressed as percentages of the corresponding controls (dashed line) as means ± s.e. of a ratio: n = number of observations. All activities after adrenalectomy are significantly different from control. A: male rats weighing 40–60 g at adrenalectomy and used between 9 and 20 days after operation, B: male rats weighing 100–125 g at adrenalectomy and used between 16 and 40 days later, C: male rats weighing 220–250 g at adrenalectomy and used between 117 and 146 days later. Tyramine was used to provide a check that adrenalectomy had affected the MAO activity in each case.

There was a significant increase in the specific activity of the rat heart MAO with all the substrates tested. Neither the age of the rats at adrenalectomy, which varied from 22 up to 55 days (i.e., 40 g up to 250 g in weight), nor the substrate used had any significant influence on the magnitude of this increase.

When rats were adrenalectomized during the summer months the increase in heart MAO activity was often delayed with all substrates except benzylamine. Table 2 shows the effect of adrenalectomy

Table 2. *Effect of adrenalectomy (ADX) in August on the metabolism of several substrates by rat heart MAO.*

Group†	MAO activity nmol (mg protein)$^{-1}$ h^{-1} Tyramine	Benzylamine	Dopamine	5-HT	β-Phenethylamine
Control 26	173 ± 24	10·1 ± 0·8	38·9 ± 6·3	125 ± 8	70 ± 10
ADX 26	185 ± 27	14·7 ± 1·6	42·5 ± 5·8	120 ± 16	81 ± 8
ADX/ control	1·07	1·45*	1·09	0·96	1·16
Control 53	384 ± 78	14·3 ± 1·7	142 ± 31	400 ± 65	N.D.
ADX 53	763 ± 148	28·2 ± 4·2	289 ± 61	659 ± 107	N.D.
ADX/ control	1·99*	1·94*	2·03*	1·65*	—

The rats from a single batch weighed 130–170 g (45 days old) at adrenalectomy, 6 control and 6 adrenalectomized rats were killed 26 days later and 5 of each group were killed 53 days later. Values are expressed as means ± s.e.m. The significance of the difference between the cardiac MAO activities of adrenalectomized and control rats was calculated by *t*-test for independent samples using the absolute values. *$P < 0·05$. ADX, adrenalectomized; N.D., not done. When similar experiments were done in winter no delay in the increase in MAO activity in the adrenalectomized animals was seen.
† With days after operation.

performed at the beginning of August. At 26 days after operation only the MAO activity, measured with benzylamine, was significantly increased compared with age-matched controls. At 53 days the MAO activity was significantly increased with all substrates.

Of the substrates used in these experiments, only benzylamine is metabolized by MAO-A and -B (and by a small proportion of a clorgyline-resistant enzyme), while the others are metabolized by MAO-A alone in the rat heart (Lyles & Callingham, 1974; 1975). Fig. 3 shows the effects of the selective inhibitor, clorgyline on the MAO activity in the hearts of the same rats as shown in Table 2, at 26 and 53 days after operation with benzylamine as substrate. At 26 days there was a significant difference between plateau values of the inhibition curves of the MAO of the hearts of the adrenalectomized and control animals, while no difference could be seen by 53 days. The observation that the plateau occurred at a higher residual MAO activity in the adrenalectomized rats would indicate that the relative proportion of MAO-B had increased (see Youdim, 1975).

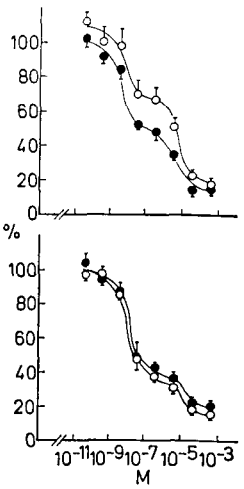

FIG. 3. The effect of clorgyline (M) on the *in vitro* activity of MAO (%) in homogenates of hearts from adrenalectomized, ●—●; and normal rats, ○—○. Rats weighing 130–170 g were adrenalectomized in August and killed, together with their controls, at 26 days, (upper graph) and 53 days after operation, (lower graph). Each point represents the mean MAO activity in the homogenates, assayed in duplicate, from 5–6 rat hearts ± s.e. of a ratio, plotted against the molar concentration of clorgyline. The difference between the plateau regions in the upper graph is significant ($P < 0·05$).

Adrenalectomy caused significant increases in the Vmax values of the heart MAO from both male and female rats (Table 3) without any change in Km, when tyramine was used as substrate.

Table 3. *The effect of adrenalectomy (ADX) on the kinetic constants of the oxidative deamination of tyramine by cardiac MAO in male and female rats.*

Group	Body wt	Days after ADX	Kinetic constants Km (× 10^5 M)	Vmax nmol (mg prot.)$^{-1}$h^{-1}
Females				
Control	169 ± 3	19	5·82 ± 2·62	183 ± 17
ADX	160 ± 3		7·13 ± 2·52	332 ± 27**
Control	203 ± 5	29	4·57 ± 0·87	218 ± 7
ADX	190 ± 4		8·42 ± 1·90	495 ± 28**
Males				
Control	184 ± 4	13	6·02 ± 1·58	121 ± 7
ADX	148 ± 3		3·30 ± 1·22	189 ± 10**
Control	296 ± 7	40	7·68 ± 1·92	526 ± 31
ADX	223 ± 6		9·83 ± 3·45	730 ± 69*

Male rats weighed 400–125 g (37 days old) and female rats 120–140 g (40 days old) at adrenalectomy. At different times after operation groups of 5 animals were killed together with their appropriate controls. Kinetic measurements were made on pooled homogenates. The substrate was used at 5 concentrations (0·1, 0·125, 0·25, 0·5 and 1·0 mM). No significant differences could be seen between the Km values of control and adrenalectomized animals, while all differences in Vmax were significant. *$P < 0·05$; **$P < 0·01$.

In a second set of experiments with male rats, but with tyramine, dopamine, 5-HT and benzylamine as substrates, significant increases in Vmax values were seen with all substrates without change in Km (Table 4).

Table 4. *Kinetic constants of the oxidative deamination of different substrates by cardiac MAO from control and adrenalectomized rats (ADX).*

	Kinetic constants			
	Km ($\times 10^5$ M)		Vmax [nmol (mg prot.)$^{-1}$h^{-1}]	
Substrate	Control	ADX	Control	ADX
Tyramine	9·63 ± 1·04	10·67 ± 2·44	289 ± 10	585 ± 45
	9·57 ± 1·72	14·68 ± 1·99	501 ± 29	1111 ± 58
Benzyl amine	9·57 ± 4·10	9·15 ± 4·31	12 ± 2	23 ± 3
	11·59 ± 6·47	12·31 ± 1·78	21 ± 4	42 ± 2
Dopamine	31·21 ± 10·28	27·45 ± 5·07	154 ± 26	274 ± 25
	17·69 ± 4·74	24·56 ± 6·99	149 ± 16	410 ± 55
5-HT	14·06 ± 2·85	11·28 ± 7·53	231 ± 18	451 ± 58
	27·95 ± 12·90	18·20 ± 6·75	587 ± 133	962 ± 121

Male rats weighed 130–170 g (45 days old) at adrenalectomy and were used at 53 days after operation. Measurements with all substrates were done on two homogenates each prepared from single hearts. Individual values are given above. The substrates were used at 4 concentrations (0·125, 0·25, 0·5 and 1 mM). No significant differences could be seen between the Km values of control and adrenalectomized animals, while all differences in Vmax values were significant ($P < 0.05$).

DISCUSSION

Unlike the other enzymes examined, the specific activities of both MAO and NCR in homogenates from rat hearts, increase with the age of the animal (Tables 1 and 3). A variety of factors could be responsible. For instance, an increase in the amount of enzyme protein, or a change in the nature of the enzyme or in the availability of its cofactor could be important. The amount of enzyme protein could itself be dependent on its rate of synthesis, on its rate of degradation or both. Previously, in the case of MAO we have shown (Della Corte & Callingham, 1977) that the rate constant for the apparent degradation of rat heart MAO falls with age. However, no direct evidence is available to show that there really is a slowing down in the rate of destruction of either MAO or NCR with age due to the very long half-lives of both enzymes.

MAO and NCR are similar in another respect: of the several enzymes that were measured, from the mitochondria, microsomes and lysosomes, only MAO and NCR increased in specific activity following adrenalectomy of the animal. The finding that the NCR total activity was increased to a lesser extent than the MAO activity first suggested that adrenalectomy could have a selective effect on one or other of the components of the NCR. Since the rotenone-sensitive NCR activity is located on the inner membrane but the rotenone-insensitive NCR, like the MAO can be found on the outer mitocondrial membrane, it was thought more likely that this latter component would be the one affected by adrenalectomy. Moreover, Okamoto (1971) has shown that treating the animal with (−)-thyroxine decreases the activities of MAO and rotenone-insensitive NCR, while increasing the activities of enzymes on the inner membrane of rat liver mitochondria. However, in our experiments, where the NCR was measured in the presence of rotenone, there was an increase in activity of the same proportion as had been found for the total NCR activity. Since there are roughly equal amounts of the two components in the rat heart, no evidence could be found to limit the effect of adrenalectomy to the outer membrane of the mitochondrion.

At present the precise mechanism responsible for the increase in the activities of MAO and NCR remains a mystery. Although there is evidence that the increase in MAO could be due to an increase in the zero-order rate of synthesis of the enzyme (Della Corte & Callingham, 1977), which is maintained for at least three months after operation, the increase in NCR activity reaches a maximum at about 15–19 days and then returns towards the control value (Della Corte, 1975). No differences can be detected between the half-lives of either MAO or total NCR from adrenalectomized rats and their appropriate controls. It would seem that, at some time after operation, the rate of apparent synthesis of NCR is increased, but this increased rate cannot be maintained, unlike that seen with MAO.

It is unlikely that a gross change in the structure of the mitochondrial membrane is involved, since SDH on the inner membrane is largely unaffected by growth or by adrenalectomy. Again, an action through the FAD cofactor is unattractive since SDH is also a flavin-dependent enzyme. It is possible that the observed increases in MAO and NCR activities that follow adrenalectomy, are due to changes in the lipid environment of the enzyme proteins; especially since Houslay & Tipton (1973) have suggested that the lipids may control the activity of MAO by an allosteric mechanism. Hulbert, Augee & Raison (1976) have shown that thyroidectomy of rats increases the unsaturation of liver mitochondrial membrane lipids, while treatment of the animal with thyroxine has the opposite effect. Adrenalectomy has been found to reduce the total phospholipid content of the myocardium (Chowdhury & Belsare, 1967). Since MAO at least, is intimately associated with the lipid components of the mitochondrial membrane, the possible importance of these com-

ponents in mediating the effects of adrenalectomy in the rat needs to be clarified.

In spite of the uncertainty surrounding the mechanism involved, the present results indicate that adrenalectomy causes an increase in Vmax values for MAO, with all substrates tested, without an effect on Km. It is reasonable to conclude that the nature of the interaction between the enzyme and its various substrates is unaltered, but its catalytic activity is increased. One is tempted to suggest that this can best be achieved by the synthesis of new enzyme with properties identical with those of the enzyme in the hearts of normal animals, since it has been shown that the MAO activities in homogenates of the hearts from control and adrenalectomized rats have very similar sensitivities to changes in pH, heat inactivation and enzyme inhibitors (Callingham & Laverty, 1973). At present we have insufficient data to be able to suggest that the action of adrenalectomy on NCR activity is mediated in a similar way. It is worth noting, however, that the only enzymes affected by adrenalectomy in these experiments, are those that increase in specific activity with the age of the animal.

Over a number of years, when only tyramine had been used as the substrate for MAO, it was noticed that the increase in MAO activity did not occur at the expected time in many of the rats adrenalectomized in the summer, although some responded normally (Della Corte, 1975). In consequence several experiments were abandoned as insufficient animals remained to test further. However, the use of several substrates and the selective irreversible inhibitor clorgyline has thrown some light on this seasonal difference. Johnston (1968) introduced the convention that is commonly used to define two forms of MAO activity that can be found in many tissues. He defined MAO-A as that activity for which 5-HT was the substrate and which was very sensitive to inhibition by clorgyline. MAO-B was less sensitive to clorgyline, and in rat liver and brain, deaminated benzylamine. Using this convention, it has been shown that the MAO activity against benzylamine in homogenates of the hearts of rats weighing about 130–170 g, was made up of roughly equal proportions of MAO-A and -B (Lyles & Callingham, 1974). When adrenalectomy was repeated in the summer with several substrates, it was found that only the activity against benzylamine was increased, at a time, which in the winter would have raised the MAO activity against all the substrates used. The increase in the benzylamine metabolizing activity at this time was about half of that seen in the winter. This, together with the difference between the clorgyline inhibition curves would indicate that, during the summer, the effect of adrenalectomy is first seen on the activity of MAO-B. About a month later, the clorgyline inhibition curves are almost identical, indicating that the proportions of MAO-A and -B in adrenalectomized and normal hearts is the same, although of course the absolute amounts of both forms are double in the adrenalectomized animals. Unfortunately, it is not possible to repeat these experiments with a substrate only attacked by MAO-B in the rat heart, as no suitable substrate has been found. All the other substrates used are almost entirely metabolized by MAO-A in this organ. It would appear likely that, in the summer, that the response of the rat heart MAO-A to adrenalectomy of the animal is delayed.

The mechanism responsible for this seasonal variation in response to adrenalectomy is again hard to identify. However, seasonal variations have been observed in the response of the heart MAO activity of the ground squirrel to adrenalectomy (Petrović & Janić, 1974). The increase did not occur at the expected time when the animals were adrenalectomized in the winter, when adrenocortical activity was at its lowest (Petrović & Janić, 1964). In man, adrenocortical activity is at its highest in the winter and also during periods of hot weather (Watanabe, 1964). It seems unlikely that changes in the environmental temperature are responsible for the observed seasonal difference in the rat in these present experiments, since the animals were kept under relatively stable conditions in the animal house. This possible relation between the initial activity of the adrenal cortex and the nature of the response of the MAO activity in the rat heart to adrenalectomy of the animal needs to be resolved. It is just possible that the season may influence the effect of adrenalectomy on the activity of the NCR as well.

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