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Footshock Affects Heart and Brain MAO and MAO Inhibitory Activity and Open Field Behavior in Rats

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LEMOINE, A. P., I. ARMANDO, J. C. BRUN, E. T. SEGURA AND M. BARONTINI. *Footshock affects heart and brain MAO and MAO inhibitory activity and open field behavior in rats.* PHARMACOL BIOCHEM BEHAV **36**(1) 85–88, 1990.—This study examined the effects, after 1 min or 2 hr, of one footshock session on the activity of MAO in rat heart and brain, the MAO inhibitory activity of these tissues, and the animal's behavior in an open field. Internal ambulation was reduced at both times; the lowest score was registered at 1 min. The number of boluses emitted during the test was higher in the group tested at 2 hr than in the other groups. One min after shocks MAO activity in heart and brain was decreased. In the heart MAO was still decreased 2 hr later, then reaching the lowest levels, while at that time, brain MAO was not different from controls. When assayed separately (MAO A and B), only the A form was found to change. MAO inhibitory activity in heart was increased at both times, the highest activity observed 2 hr after footshock. Brain MAO inhibitory activity was increased only in the 1-min group. Ex vivo competition experiments with clorgyline suggested presence in vivo of a reversible MAO inhibitor. The time-dependent response to stress of both MAO activity and MAO inhibitory activity in the tissues correlates with the responses observed in the open field test. These findings suggest that the observed biochemical changes might be related to increased autonomic activity and to the state of fear and anxiety evoked by the stressful procedure.

Stress Inescapable footshock MAO MAO inhibitory activity Open field Behavior

ALTHOUGH mechanisms other than enzyme concentration regulating MAO activity in vivo have been postulated, evidence for short-term regulation of the enzyme is scarce. Rapid changes in MAO activity have been reported in relation to circadian rhythms in the hypothalamus (13,30) and in specific areas of the brain stem (8), and following stressful stimulation. Cold decreased MAO activity in rat brown scapular fat (6), while chronic environmental stress induced changes in MAO activity in some brain regions (22). We recently demonstrated that cold-restraint induced decreased MAO activity in heart and kidney, accompanied by an increase in the MAO inhibitory activity (3).

Two types of endogenous MAO inhibitors have been described. Becker *et al.* (4) and Isaac *et al.* (20) reported peptide molecules producing MAO inhibition. The levels of the latter were increased after electroconvulsive shock (20). The other is a nonpeptide, low molecular weight, neutral molecule (or class of molecules) which has been called tribulin (12, 17, 28). Its levels were increased in some tissues after cold restraint (3,5), repeated isolation (2), and immobilization (5).

Stressful procedures lead to long-term changes in both physiological and behavioral responses. The proactive interfering effects of stressful inescapable stimulation on subsequent escape learning have been described (25).

This paper investigates the time course of biochemical and behavioral changes induced by a stressful procedure. We report the effects, at two different intervals, of one inescapable footshock session on rat heart and brain MAO activity and MAO inhibitory activity, and those elicited on locomotor and exploratory activity tested on an open field.

METHOD

Animals

Adult naive male Sprague-Dawley rats weighing 250–300 g and kept under standard conditions were used. Experimental animals were subjected to one session of 10 inescapable footshocks (5 mA, 3 sec duration) presented at fixed 45-sec intervals.

Control animals were subjected to the same manipulation as experimental rats (i.e., placed in the test cage for 9 min) but not shocked.

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At two intervals (1 min and 2 hr) after the experimental session, groups of animals were either tested on an open field or sacrificed by cervical dislocation. Hearts and brains were rapidly removed, placed on ice and stored frozen until assayed.

Open Field Test

The test was carried out as described by Bures *et al.* (7). Animals were placed in one corner of an homogeneously illuminated (50 W) open field (1 × 1 × 0.4 m). The floor had 25 identical squares, 16 of them peripheral or external (i.e., adjacent to walls) and 9 central or internal (nonadjacent to walls). Two activities were recorded: defecation and ambulation. The latter was scored as external and internal square ambulation. Each crossing of a dividing line with at least the two forelegs was scored; this was considered internal ambulation when the animal entered an internal square, and external ambulation when it entered an external one. The test lasted 5 min. The number of boluses emitted during the test was also recorded.

MAO Inhibition Assays

For each experiment, tissues collected from 4 rats were pooled, weighed and homogenized (20–50%) with an Ultraturrax in cold 2 N HCl. Homogenates were centrifuged (0–4°C) for 10 min at 7000 rpm. Supernatant was transferred to glass tubes and extracted into 2 vol. redistilled ethyl acetate. After centrifugation the organic layer was carefully removed and dried under N_2. Blanks containing equal volumes of 2 N HCl were also extracted into ethyl acetate and put through the same procedure. Residues were taken up in 220 μl of 100 mM phosphate buffer pH 7.4 and the MAO inhibitory activity was tested as described by Glover *et al.* (17). Briefly, 100 μl aliquots were incubated (30 min at 37°C) with 20 μl of MAO preparation (1% w/v homogenate of rat liver) and 10 μl of ^{14}C-tyramine (specific activity 58.9 mCi/mmol, New England Nuclear) diluted with unlabelled tyramine to reach a final concentration in the incubation mixture of 83 μM. The radioactive product formed was extracted into 3 ml of toluene:ethyl acetate (1:1) and after centrifugation the organic phase was transferred to vials for liquid scintillation spectrometry. The MAO inhibitory activity was expressed in units/g of wet tissue. A unit of activity was arbitrarily defined as one producing 25% of inhibition in the test assay system (1).

MAO Tissular Activity Assay

Tissues were homogenized in 3–5 vol. 100 mM phosphate buffer pH 7.4; centrifuged (0–4°C) at 5000 rpm for 10 min and 20 μl aliquots of supernatant were incubated with ^{14}C-tyramine (final concentration 83 μM), ^{14}C-5 hydroxytryptamine (final concentration 153 μM) or ^{14}C-phenylethylamine (final concentration 5 μM). Assay procedure was similar to MAO inhibition test.

Ex Vivo Evaluation of MAO Activity

A group of experimental animals were injected (IP) 1 min after shock session, with clorgyline diluted in 0.2 ml of saline, 25 mg/kg. Control animals were injected with the same dose of clorgyline but not subjected to inescapable shocks. Both groups were sacrificed 120 min after injection. MAO activity was determined in extensively washed homogenates from hearts and brains as described above.

Statistical Analysis

Statistical analysis used Student's *t*-test for unpaired data, or one-way ANOVA followed by pair-wise comparison test.

RESULTS

Results from the open field test are shown (Table 1). Internal ambulation was significantly lower both 1 min and 2 hr after the shock session compared to the control group ($p<0.01$ for both). This score was also significantly lower in the animals tested 1 min after the session compared to those tested 2 hr later. The number of boluses emitted during the test was significantly lower at 1 min compared to controls ($p<0.01$). However, it was significantly

TABLE 1

EFFECTS OF ONE INESCAPABLE FOOTSHOCK SESSION ON OPEN FIELD TEST

	Internal Ambulation	Defecation
Control (n = 10)	6.25 ± 1.25	1.45 ± 0.20
Footshock		
1 min (n = 10)	0.37 ± 0.24*†	0.10 ± 0.09*
2 hr (n = 10)	1.72 ± 0.38*	2.60 ± 0.53‡

Internal ambulation is expressed in number of crossings/session; defecation is expressed in number of boluses emitted/session; values are expressed as mean ± SEM.

*$p<0.01$ vs. control; †$p<0.01$ vs. 2 hr; ‡$p<0.05$ vs. control and $p<0.01$ vs. 1 min.

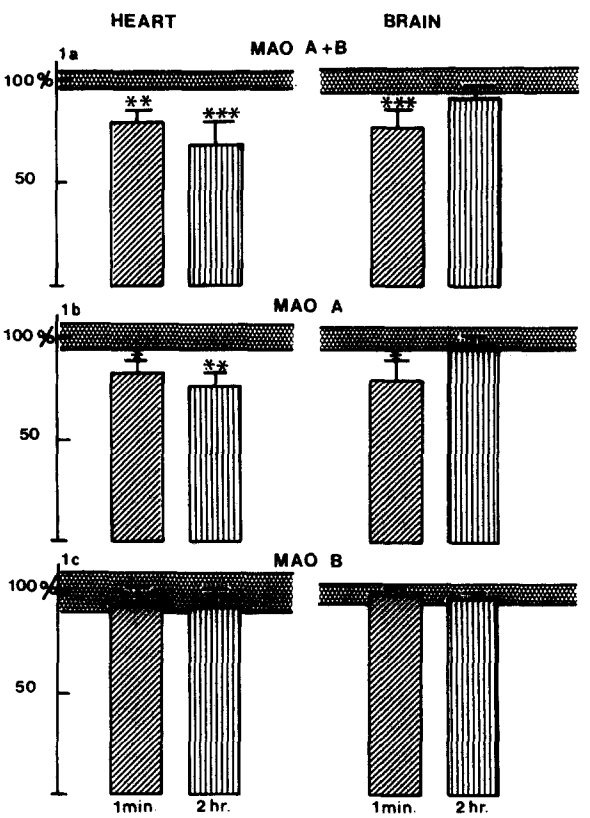

FIG. 1. Effects of one inescapable footshock session administered 1 min (hatched bars) or 2 hr (straight bars) before sacrifice on brain and heart MAO activity. Controls: cross-hatched bars. Results are expressed in percentages of control value taken as 100% (mean ± SEM of 6 experiments). *$p<0.05$; **$p<0.02$; ***$p<0.01$.

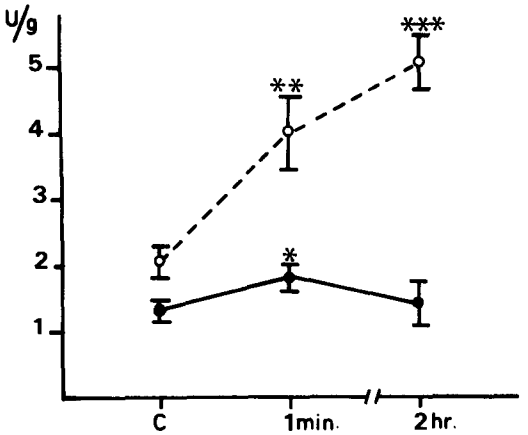

FIG. 2. Effects of one inescapable footshock session administered 1 min or 2 hr before sacrifice on heart (dashed lines) and brain (solid lines) MAO inhibitory activity. Values are expressed as mean ± SEM of 5 experiments. C = controls. *$p<0.05$; **$p<0.02$; ***$p<0.01$.

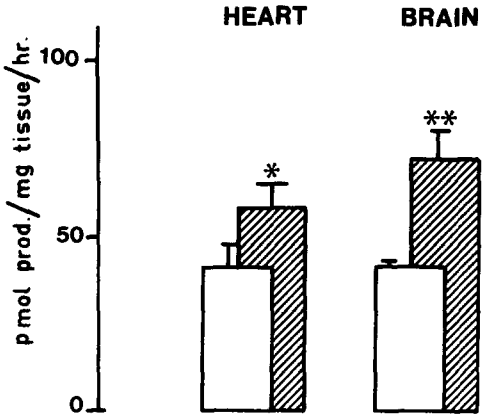

FIG. 3. Effect of clorgyline 25 mg/kg IP on MAO A activity in heart and brain in controls (open bars) and footshocked animals (hatched bars). Drug was administered 1 min after footshock. All animals were sacrificed 120 min after drug administration. Results are expressed as mean ± SEM of 6 experiments. *$p<0.05$; **$p<0.01$.

higher at 2 hr compared to both controls ($p<0.05$) and animals tested at 1 min ($p<0.01$).

The effects of one inescapable footshock session, 1 min or 2 hr before sacrifice, on brain and heart MAO activity are shown (Fig. 1). Levels of MAO activity in controls for both groups showed no significant differences, and were pooled for statistical analysis. Figure 1a shows activity of MAO determined using tyramine, a substrate for both A and B forms of the enzyme. In the heart, enzyme activity was significantly decreased at 1 min ($p<0.02$) and 2 hr ($p<0.01$) after the session. Decrease in MAO activity was greater after two hr (30%) than after 1 min (19%). However, MAO A + B levels in the experimental groups were not statistically different. In the brain, footshock also induced a decrease in MAO activity, but was statistically significant only after 1 min ($p<0.01$).

MAO-A activity assayed with 5-hydroxytryptamine substrate was decreased in heart 1 min and 2 hr after the session, with lowest levels at 2 hours (Fig. 1b). This effect in the brain was only significant after 1 min ($p<0.05$). Conversely, MAO-B activity, assayed with phenylethylamine substrate, was unchanged in heart and brain at both intervals (Fig. 1c).

Figure 2 shows MAO inhibitory activity in hearts and brains of footshocked and control rats: in heart it was significantly increased at both intervals ($p<0.02$ and $p<0.01$ for 1 min and 2 hr respectively); the highest increment (150% over control levels) was observed 2 hr after shock. A significant increase in brain MAO inhibitory activity was only found after 1 min ($p<0.05$); the levels 2 hr after shock were similar to control values.

Administration of clorgyline 1 min after the experimental session resulted in a 90% decrease in MAO activity in hearts and brains from control and footshocked animals (data not shown). Remaining MAO-A activity in homogenates of both tissues, assayed with 5-hydroxytryptamine substrate (Fig. 3), was significantly higher in brains ($p<0.01$) and hearts ($p<0.05$) from footshocked animals when compared to controls.

DISCUSSION

Our results show that one inescapable footshock session induced clear modifications in the open field test. A significant decrease of internal ambulation was observed both 1 min and 2 hr after shock. These results concord with the literature on learned-helplessness (23). Changes in internal ambulation are related to the animal's state of fear/anxiety (24). The slight but consistent recovery of exploratory activity observed in animals tested 2 hr after shock suggests that the emotional effects of the stress procedure lessen with time.

Defecation score was below control score one min after footshock, while after two hr it was significantly increased. The effect after one min can be explained by the high number of boluses emitted during shock. Increases in defecation during open field tests have been attributed to enhanced autonomic activity (19).

We found that inescapable footshock induced a time-dependent decrease in MAO activity in the two tissues assayed. Brain MAO activity was lowest 1 min after shock, while in heart it was lowest after 2 hr. Footshock-induced changes in brain MAO activity were immediate and temporary: 2 hr after stress, enzyme activity regained control values. Results showed that changes in total MAO activity were due only to changes in the A form. The stress-induced decrease in MAO activity found is in agreement with several previous reports (3, 6, 22). Changes in both heart and brain MAO activity were accompanied by opposite changes in the tissue MAO inhibitory activity. A rapid and short-lasting increase in this inhibitory activity was observed in brain, while in heart it was significantly increased at both intervals. This also agrees with a previous report that in rat heart, cold-restraint resulted both in a decrease in MAO activity and a concomitant increase in tissue MAO inhibitory activity (3).

To assess the enzyme's functional state in vivo, we have made competition experiments with clorgyline (an irreversible MAO-A inhibitor) (18). This procedure resulted in less brain and heart irreversible inhibition in footshocked rats compared to controls, suggesting a reversible MAO inhibitor in vivo. Similar results have been reported in brains of cold-restrained rats using phenelzine (15).

We have attributed tissue MAO inhibitory activity to tribulin-like material, with a distinct regional distribution in rat tissues (1), and differentially increased after stress (1–3, 5). Tribulin appears to bind to benzodiazepine receptors (9, 12, 28), and has been shown increase in both animals and humans in conditions of stress and anxiety. In the rat, its urinary output is raised after 2 hr of cold restraint stress, but attenuated by benzodiazepine pretreatment (14), while in humans tribulin urinary output was increased in

general anxiety disorder (10), alcohol withdrawal (27), benzodiazepine withdrawal (26), and lactate-induced panic attacks (11). Tribulin has also been found naturally in other species such as pigs (29) and mice (unpublished data). Recently, Glover *et al.* reported that purified tribulin from normal human urine has been identified as isatin. Considerable amounts of isatin were also found in both rat brain and heart (16). In vitro, isatin is a potent MAO inhibitor, more active against MAO-B than MAO-A. We have seen no effect on MAO-B activity: we could assume either that tissue MAO inhibitory activity is not identical to isatin [a possibility raised by Glover *et al.* (16)], or that the two forms of the enzyme are differently located. It is accepted that in the heart, intraneuronal MAO is of the A form, while in the brain MAO-A is contained in catecholaminergic neuronal areas (21,31). Thus, under stress, tribulin could be involved in the regulation of catecholaminergic neurotransmitter levels.

The time-dependent response to stress in both MAO activity and tribulin-like activity in tissue matches responses observed in the open field test. It may be that the rise in brain tribulin is related to the state of fear and anxiety evoked by the stressful situation. This hypothesis is supported by the changes observed in the open field internal ambulation. However, whether stress-induced changes in both tribulin and exploration requires an anxiety-derived interpretation remains open to question. It is tempting to speculate that the sustained increase in heart tribulin is related to the increased autonomic activity evidenced by the increment in defecation scores.

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