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# The Effect of Imipramine and Lithium on "Learned Helplessness" and Acetylcholinesterase in Rat Brain

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GEOFFROY, M., K. TVEDE, A. V. CHRISTENSEN AND J. S. SCHOU. *The effect of imipramine and lithium on "learned helplessness" and acetylcholinesterase in rat brain.* PHARMACOL BIOCHEM BEHAV **38**(1) 93–97, 1991.—The effect of short- and long-term treatment with imipramine and lithium on shock stress-induced escape failures in a shuttlebox (the "learned helplessness" model of depression) was investigated in rats. Acetylcholinesterase (AChE) activity was measured in the frontal cortex, hippocampus and striatum after the shuttlebox test. Imipramine was found to normalize escape behavior, whereas lithium further aggravated escape behavior. No correlation was found between escape behavior and AChE activity in the three brain areas investigated. However, a significant decrease in AChE activity in striatum was found in rats exposed either to shock stress and no drug treatment or to drug treatment and no shock stress. In rats exposed to the combination of shock stress and drug (imipramine or lithium), a slight or no decrease of AChE activity occurred. Exposure to shock stress alone produced no changes in AChE activity in the hippocampus and frontal cortex. In conclusion, lithium did not have an antidepressant effect on "learned helplessness" and AChE activity was not correlated to escape behavior. However, both imipramine and lithium normalized the decreased level of AChE activity in striatum in rats exposed to shock stress.

Shock stress Learned helplessness Acetylcholinesterase Lithium Imipramine Striatum Hippocampus Frontal cortex Rat

EXPOSURE to a session of inescapable shock (IS) induces a high number of escape failures in rats when tested four days later in a two-way shuttlebox (18). It has been proposed that the behavioral and physiological changes induced by IS parallel symptoms found in depressed humans (29), and the paradigm has become an experimental model of depression called "learned helplessness." It has been shown that the number of escape failures decrease to a normal level after subchronic (but not acute) treatment with imipramine or other tricyclic antidepressants (TCAs), monoamine oxidase inhibitors and electroconvulsive shocks (ECT) (4, 19, 24). However, the model lacks pharmacological specificity since acute treatments with nonantidepressants such as amphetamine or scopolamine also are effective (1). Furthermore, some new atypical antidepressants seem to be ineffective (11).

Experimental stress has been shown to produce muscarinic receptor supersensitivity, presumably due to presynaptic hypofunction (5, 12, 26). Muscarinic supersensitivity is also produced by treatments with TCAs which block muscarinic receptors (6,25). Thus, both experimental stress and TCAs produce supersensitivity of muscarinic receptors. In addition, experimental stress produces downregulation of beta-adrenergic receptors, an effect also characteristic of TCAs (22). It is remarkable that induction of a "pathological" state with stress and its "treatment" with TCAs have the same supersensitizing effect on muscarinic and adrenergic receptor systems. In order to gain further insight into the changes which follow after stress and antidepressant treatment we have investigated the effect of short- and long-term treatment with imipramine and lithium in "learned helplessness." Imipramine and lithium seem to have opposite effects on the activity of the cholinergic system. TCAs decrease cholinergic activity (6) and lithium increases cholinergic activity in the cortex, hippocampus and striatum of rat brain (14,16). Studies on the effect of ECT (also an antidepressant treatment) have shown that acetylcholine (9) as well as acetylcholinesterase (AChE) activity (2) decrease in rat brain after ECT. We have measured AChE activity in the striatum, hippocampus and frontal cortex immediately after testing the rats in the shuttlebox escape test.

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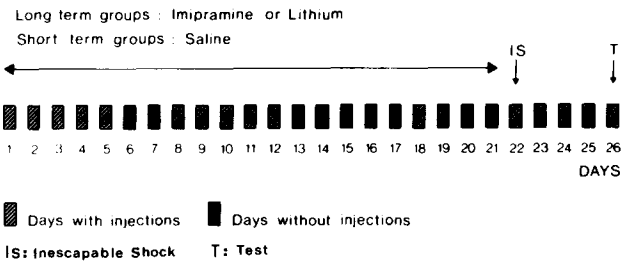


FIG. 1. Experimental schedule for long-term and short-term groups. Long-term drug groups were treated with imipramine or lithium during the 21 days preceding the session of inescapable shock (IS). Short-term drug groups were injected with saline during these 21 days. In the period between the exposure to IS and the shuttlebox test (T), both short- and long-term drug groups recieved imipramine or lithium.

## METHOD

### *Animals*

Male Wistar rats (Møllegaard, Havdrup) weighing 200–230 g were housed in pairs in standard macrolon cages with free access to standard rat chow and tap water. The lithium-treated animals were given tap water containing 10 mM NaCl to avoid toxic effects (27). Room temperature was 19–22°C and lights were on from 6 a.m. to 6 p.m. The experiments started one week after adaptation to housing conditions. Seventy-two rats were randomly assigned to 6 short-term groups and 6 long-term groups ($n=6$).

### *Injection Schedules*

The long-term groups were injected with imipramine or lithium IP twice a day for 21 days (see Fig. 1). In the same period the short-term groups were injected with saline. On day 22 both short- and long-term groups were injected 2 h after exposure to a session of inescapable shock ($IS^+$), or a no-shock control session ($IS^-$); drug treatment continued until day 26 where the last injections of imipramine or saline were given 2 h before the escape test in the shuttlebox. Lithium treatment was discontinued 12 h before the shuttlebox test and replaced by saline 2 h before testing (in order to avoid eventual accumulated toxic effects due to high levels of serum-lithium combined with exposure to shock stress during the test). No adverse effects of the drug treatments were observed during the 26 days. As expected, long-term lithium treatment increased water intake and body weight more relative to the other treatments.

### *Drugs*

The doses of drugs injected (twice a day at 9 a.m. and 5 p.m.) were: 2 ml 0.9% saline or 2 ml 0.2 M LiCl [serum-Li elimination curve, see (23)], or 12.5 mg/kg imipramine-HCl in a volume of 2 ml/kg.

### *Inescapable Shock (IS)*

Four Lafayette two-way shuttleboxes were used in the application of IS and for the test of escape failures. Each box consisted of two stainless steel chambers separated by a shuttle partition (6 cm high) with an 18 bar grid floor ($30\times20\times20$), that was pivot-mounted to drive location sensors. Two 28-V houselights were fitted to the ceiling. An electrified bar with a different polarity than that of the shuttle partition was installed on the top lip of the partition to prevent the rat from balancing on the edge and avoid shock.

The walls of the box were also electrified. A one-way glass mirror along the front walls allowed observation. For the IS session a partition wall was inserted in the middle of the shuttleboxes, allowing two rats in each box to be subjected to IS at the same time. The IS session lasted 30 min (houselight on). Scrambled unsignalled electric shock (1.0 mA) was delivered through the grid floor using a computer-controlled schedule at random. A period of SHOCK-ON or SHOCK-OFF was $\geq 0.5$ s and the number of periods was randomized by a computer so that the total SHOCK-ON time was 15 min (50%) of the session time. This random schedule was to ensure that shock termination was independent of ongoing behavior and gave different shock presentations to each animal. The shock source was a Coulbourn model E13-08 shock generator. IS control groups were subjected to the same procedure except that the shock generator was turned off during the IS session.

### *Shuttlebox Test*

The shuttlebox test used was in accordance with the procedure described in (17). The shuttlebox test took place on day 26, between 8 and 12 a.m. After an initial 5-min habituation (houselight on), a total of 25 nonsignalled escape trials were presented (shock intensity 0.8 mA). In the first 10 trials the rats were required to change side once (FR1), and in the following 15 trials the rats had to change side twice (FR2) in order to terminate shock. The shock was continuously present during each trial which lasted 15 s max. Failure to respond during the 15 s was designated an escape failure. The intertrial time was 20 s during which a response had no programmed consequences. Data acquisition was computerized.

### *Measurement of AChE Activity*

Immediately after the shuttlebox test, the rat was killed by decapitation, the brain removed, cold dissected, and frozen at −20°C. AChE activity was assayed by the method in (8).

### *Statistical Analysis*

Statistical analysis of the behavioral data was carried out by means of the STATGRAPHICS statistical package. The nonparametric tests used were Kruskal-Wallis followed by Wilcoxon. Data from FR2 escape trials are shown. For statistical analysis of AChE activity data Student's *t*-test was used. Because of the high sensitivity of the assay and very small variances within groups, a number of 3–4 was sufficient to show significant differences. Values of AChE activity are presented as means ± SEM.

## RESULTS

### *Escape Behavior*

Exposure to IS ($IS^+$) significantly increased the number of escape failures in the short- as well as in the long-term group (Fig. 2). After short- and long-term treatment with lithium the number of escape failures induced by $IS^+$ was increased ($p<0.01$). Lithium did not influence the number of escape failures in the control groups ($IS^-$ Li). In the long-term group exposed to $IS^+$ and imipramine not one escape failure was produced, i.e., imipramine counteracted the effect of $IS^+$ ($p<0.01$). In the short-term imipramine group, the decrease in the number of escape failures failed to reach statistical significance.

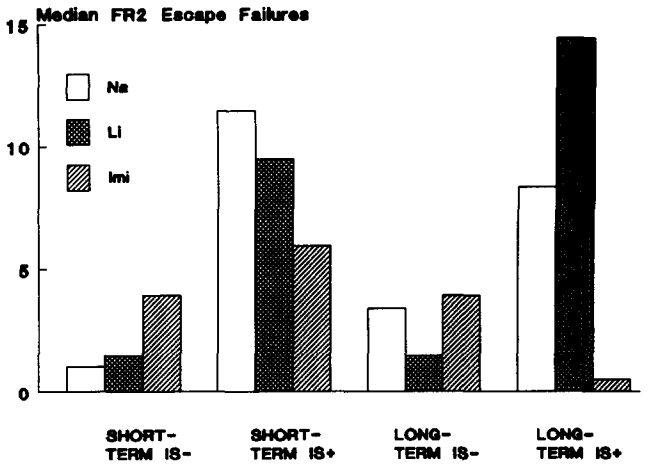


FIG. 2. Median No. of escape failures in the shuttlebox test (n = 6). $IS^-$ = no exposure to inescapable shock; $IS^+$ = exposure to inescapable shock. Short-term groups were treated with drugs for 4 days; long-term groups were treated 26 days and IS was administered 4 days before the shuttlebox test. The range of the medians were (left to right): (0–11) (0–14) (0–14); (0–15) (0–15) (0–14); (0–9) (0–14) (0–11); (1–12) (10–15) (0–0). Short-term imipramine $IS^+$ vs. $IS^-$ failed to reach statistical significance. Long-term NaCl $IS^+$ vs. NaCl $IS^-$: $p<0.05$. For all other $IS^+$ groups vs. $IS^-$ groups given the same drug treatment: $p<0.01$.

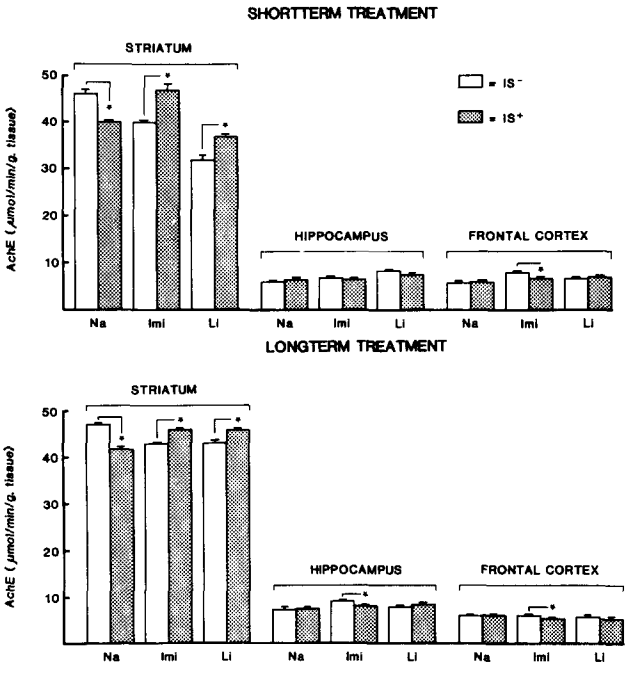


FIG. 3. Values of AChE are presented as means ± SEM from n = 4 (short-term groups) and n = 3 (long-term groups). $IS^-$ = no exposure to inescapable shock; $IS^+$ = exposure to inescapable shock. Na = saline; Imi = imipramine; Li = lithium.

*Changes in AChE Activity*

AChE activity was measured in the striatum, hippocampus and frontal cortex immediately after the rat had finished the shuttlebox test.

*Striatum*

In the saline-treated group, a fall in AChE activity was seen in the striatum after $IS^+$ in the short-term (13%) and long-term (11%) groups (see Fig. 3). In both short-term drug-treated groups not exposed to $IS^+$ a similar decrease in AChE activity was seen: short-term imipramine decreased AChE by 14%, whereas short-term lithium decreased AChE activity by 31% compared to the corresponding $IS^-$ saline control groups. In long-term drug-treated groups not exposed to $IS^+$ the decrease in AChE activity was less than in the short-term groups (imipramine: 9%; lithium: 9%). However, independent of prior exposure to IS, AChE was increased in the long-term imipramine and lithium groups compared to the short-term groups (see Table 1).

Exposure to both $IS^+$ and imipramine treatment (short- or long-term) produced no significant changes in AChE activity in striatum. Exposure to both $IS^+$ and lithium treatment had a similar effect (no change in AChE activity) in the short- and long-term groups, although a 20% decrease in AChE activity was still present in the short-term group. Short-term lithium treatment induced a decrease in both $IS^-$ and $IS^+$ groups compared to the saline or imipramine-treated groups (Table 1).

*Hippocampus*

$IS^+$ alone did not induce changes in AChE activity in the hippocampus (Fig. 3). Imipramine treatment induced no changes in AChE activity in the short-term group. AChE activity was increased in the $IS^-$ long-term imipramine group compared with the $IS^-$ saline control group. Treatment with $IS^+$ and imipramine together induced a 12% decrease in AChE activity compared with the $IS^-$ imipramine group. In the short-term lithium group which was not exposed to shock stress ($IS^-$ Li), AChE activity was increased compared with the $IS^-$ saline control group. When the rats were exposed to lithium treatment and $IS^+$, the AChE activity showed a tendency to decrease compared with $IS^-$ Li in the short-term group. Independent of exposure to IS long-term saline and imipramine treatment increased AChE compared to the respective short-term treatments (Table 1).

*Frontal Cortex*

In frontal cortex, no change in AChE was induced by $IS^+$ alone (see Fig. 3). In the short-term imipramine-treated group not exposed to IS AChE activity was increased by 36% compared with the short-term saline-treated group, whereas in the long-term imipramine group ($IS^-$ Imi), no increase was found. In groups exposed to both $IS^+$ and imipramine treatment ($IS^+$ Imi), a decrease in AChE activity was seen compared with $IS^-$ Imi (20% in the short-term group, 12% in the long-term group). Lithium treatment induced no significant changes in AChE activity in the frontal cortex. Both imipramine and lithium decreased AChE activity in frontal cortex in long-term-treated groups compared to short-term-treated groups, independent of previous exposure to shock stress (Table 1).

## DISCUSSION

The present study confirms that inescapable shock (IS) increases the number of escape failures in the shuttlebox test. This increase in escape failures was counteracted by long-term imipramine treatment and showed a tendency to be decreased by short-

TABLE 1

SHORT-TERM VS. LONG-TERM TREATMENT ACETYLCHOLINESTERASE ACTIVITY (μmol/min/g TISSUE)

| Drug | | Striatum | | Hippocampus | | Frontal Cortex | |
|---|---|---|---|---|---|---|---|
| | | $IS^-$ | $IS^+$ | $IS^-$ | $IS^+$ | $IS^-$ | $IS^+$ |
| NaCl | (S) | 46.1 ± 0.9 | 40.2 ± 0.5 | 6.1 ± 0.1 | 6.4 ± 0.3 | 5.8 ± 0.3 | 6.1 ± 0.2 |
| | (L) | 46.9 ± 0.2 | 41.7 ± 0.5 | 7.3 ± 0.4* | 7.4 ± 0.3* | 5.9 ± 0.2 | 6.1 ± 0.4 |
| Imi | (S) | 39.9 ± 0.5 | 46.9 ± 1.2 | 6.7 ± 0.3 | 6.4 ± 0.1 | 7.9 ± 0.1 | 6.7 ± 0.3 |
| | (L) | 42.8 ± 0.1* | 45.8 ± 0.2* | 8.9 ± 0.1* | 8.0 ± 0.4* | 6.0 ± 0.1* | 5.3 ± 0.1* |
| Li | (S) | 31.9 ± 1.0 | 37.1 ± 0.5 | 8.2 ± 0.3 | 7.6 ± 0.3 | 6.7 ± 0.3 | 7.1 ± 0.2 |
| | (L) | 42.9 ± 0.6* | 45.8 ± 0.3* | 7.8 ± 0.2 | 8.3 ± 0.4 | 5.8 ± 0.2* | 5.3 ± 0.4* |

Values are mean activities ± SEM: $IS^-$ = no inescapable shock, $IS^+$ = exposure to inescapable shock. NaCl = saline, Imi = Imipramine, Li = Lithium. (S) = short-term group: (L) = long-term group.

*$p<0.05$ with Student's $t$-test.

term imipramine treatment. Short-term lithium treatment had no influence on the shock stress-induced increase in the number of escape failures, but long-term lithium treatment increased the number of escape failures to a maximum. This increase in escape failures may be explained by an increase in the activity of the cholinergic system induced by lithium (14), since it has previously been shown that increased cholinergic activity following an injection of a cholinergic agonist mimics the effect of IS on escape behavior (1). Interestingly, lithium had no effect on escape behavior in groups which were not exposed to IS. This shows that an interaction of stress and lithium is required to influence escape behavior. A recent study has shown that the influence of lithium on the phosphoinositide second messenger system is most pronounced when the phosphoinositide system is already actively stimulated (20). An effect of lithium which depends on ongoing neuronal activity could explain its different behavioral effects in stressed and nonstressed rats.

It is difficult to discuss whether the effect of imipramine on escape behavior also depends on exposure to IS, since a decrease in the number of escapes failures, in order to be significant, would have to fall below the baseline. However, the total absence of escape failures in the long-term imipramine group exposed to $IS^+$ suggests that imipramine has a more activating effect in the $IS^+$ group than the $IS^-$ group.

Before discussing the AChE activity results it should be mentioned that AChE in the brain is primarily localized to areas containing cholinergic neurons (28), where it inactivates synaptic acetylcholine. However, AChE is also present in areas not containing cholinergic neurons and seems to exist in certain regions beyond requirements (13). Frontal cortex, hippocampus and striatum are areas where the levels of AChE activity seem to be related to the amount of acetylcholine (3,13). These areas are of interest in the present context because projections between frontal cortex and the hippocampal system seem to be involved in "learned helplessness" (15,21). Striatum, which is an area extremely rich in acetylcholine and AChE activity, is involved in extrapyramidal control of motor activity (7) and thus in escape performance. Since striatum was the only area where IS alone induced changes in AChE activity, the following discussion will mainly focus on data from this area.

It can be concluded that no correlation between the changes in AChE activity in striatum and the escape behavior of the different groups of rats was present. However, the AChE activity data is interesting because a) lithium and imipramine had a parallel influence on AChE activity in striatum, and b) the influence of imipramine and lithium on AChE depended on whether or not the animal had been exposed to IS. Drug treatment alone caused a decrease in AChE activity in the striatum, just as IS alone caused a decrease in AChE. However, drug treatment combined with IS yielded "normal" levels of AChE activity. This shows that the changes induced by a drug treatment (not only on a behavioral parameter, escape failures, but also on a biochemical parameter, AChE activity) can depend on the condition of the organ/organism. A study by (10) has shown that another biochemical parameter varies depending on exposure to stress: nifedipine, a calcium channel blocker, influences binding to the calcium channel site differently in rats exposed to $IS^+$ and to $IS^-$. It is possible that many drug effects depend on the state of activity of a given organ in agreement with the notion of homoeostasis.

Studies investigating the effect of ECT on acetylcholine and AChE (2,9) have shown that ECT induces a decrease in both parameters. In the present study the treatments induced a decrease in AChE on their own, whereas the combination of stress and drug treatment paradoxically seemed to maintain a normal AChE activity level. In general, long-term treatment with imipramine or lithium significantly changed AChE activity compared to short-term treatment whether or not the animal had been exposed to shock stress. In striatum AChE activity was increased, whereas in frontal cortex AChE activity was decreased. The finding in hippocampus that long-term saline treatment increased AChE activity is surprising.

In conclusion, changes in AChE activity in striatum did not correlate with escape behavior. This does not imply that the cholinergic system is without influence on escape behavior or the effects of antidepressants, but only shows that no simple correlation is present. Imipramine and lithium showed parallel effects on AChE in striatum, but in frontal cortex and hippocampus the pattern of AChE changes were not parallel in imipramine- and lithium-treated rats. However, the present study does not permit a discussion of the significance of these changes. The different effect of imipramine and lithium on escape behavior and AChE activity in stressed and nonstressed rats indicates that the biochemical effects of the drugs depend on the state of the organism. This finding questions the relevance of studying therapeutical drug effects in healthy organisms, experimental induction of a "pathological" state may prove to be a more fruitful approach.

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