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THE INVERSE CHANGES OF MOUSE BRAIN ORNITHINE AND *S*-ADENOSYLMETHIONINE DECARBOXYLASE ACTIVITIES BY CHLORPROMAZINE AND IMIPRAMINE

DEPENDENCE OF ORNITHINE DECARBOXYLASE INDUCTION ON β-ADRENOCEPTORS

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Abstract—Intraperitoneal injection of chlorpromazine and imipramine increases mouse brain ornithine decarboxylase but decreases *S*-adenosyl-L-methionine decarboxylase activity. Maximal effect was obtained 6–8 hr after treatment at which time single dose of chlorpromazine (50 mg/kg) stimulated ornithine decarboxylase activity 7-fold and decreased *S*-adenosylmethionine decarboxylase activity to 50% from the control level. Correspondingly, ornithine decarboxylase activity was 5.5 times higher than the control value and *S*-adenosylmethionine decarboxylase activity about 40% from that after imipramine injection (80 mg/kg). The possible dependence of the enzyme responses on adrenergic receptors was studied using α-adrenoceptor antagonist, phentolamine, and β-adrenoceptor antagonist, propranolol, concurrently with chlorpromazine and imipramine. The stimulation of ornithine decarboxylase but not the inhibition of *S*-adenosylmethionine decarboxylase could be abolished by propranolol (10 mg/kg), whereas phentolamine (10 mg/kg) slightly increased ornithine decarboxylase activity even when given alone. This suggests that β- but not α-adrenergic mediation is involved in the stimulation of mouse brain ornithine decarboxylase activity and that brain ornithine and *S*-adenosylmethionine decarboxylase activities are independently regulated. When chlorpromazine and imipramine were tested *in vitro*, both of them turned out to have an inhibitory effect on *S*-adenosylmethionine decarboxylase. The former caused 50% inhibition at a concentration of 1 mM and the latter at 2 mM. Preliminary tests suggest that the type of inhibition is noncompetetive for both of them.

The fact that there is a relatively high concentration of polyamines, spermidine and spermine, in adult mammalian brain, has given rise to a number of studies aimed to investigate their possible central functions. The results have shown that polyamines have neuropharmacological effects and possible roles in central synaptic transmission [reviewed 1, 2]. The metabolic and regulatory relations of polyamines to the inhibitory neurotransmitter, 4-aminobutyric acid (GABA) [reviewed 2, 3] are of particular interest. The cerebral polyamine synthesis appears to be sensitive to different hormones [4–8] and drugs [9–14]. Many of the hormonal effects are obviously related to the various roles of polyamines in growth and cellular proliferation [reviewed 15], and some of the drug effects [9–11] are associated with the relations between polyamines and GABA. The rest of the studies describing drug effects on cerebral polyamines metabolism [12–14] may all be regarded to involve the catecholaminergic functions of the CNS.

The above-mentioned effects of hormones and drugs on cerebral polyamine metabolism are most prominent in the increased activity of L-ornithine decarboxylase (EC 4.1.1.17, ODC), the regulatory enzyme of polyamine synthesis. The changes in the activity of *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50, SAM-DC) are more occasional and not so considerable. Among mammalian enzymes these decarboxylases have uniquely short half-lives, i.e. 10–20 min [16] and 20–60 [17], respectively. This enables their activities to be controlled through *de novo* synthesis and through the changes in their half-lives. It has been suggested in two recent reviews [18, 19] that ODC activity may be a useful tool as an index when the trophic effects of drugs and hormones on target tissues are to be evaluated. We decided to apply this kind of approach to obtain new information on the mechanisms of action of psychoactive drugs. This type of research is envisaged to bring also some insight into the roles of polyamines in the CNS.

Chlorpromazine and imipramine, with their derivatives, form an interesting pair of psychotrophic drugs, since in spite of the close structural analogy, they have definitely different neuropharmacological properties. Because both of the drugs have well-established but different effects on catecholaminergic functions [see e.g. 20–23], which in turn have been linked to cerebral polyamine metabolism [12–14], they were regarded to be particularly suitable for this study.

MATERIALS AND METHODS

Animals. The study was carried out using albino mice of the NMRI strain, weighing 30 ± 2 g. The

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animals had free access to water and food (standard pelleted mouse diet by Hankkija Oy, Turku, Finland), and they were housed in quarters with automatically timed, regularly alternating 12 hr periods of light and darkness at 20°. All animals were killed by cervical disclocation between 8 and 10 a.m. to eliminate the influence of diurnal variation on the enzyme activities.

Drug treatments. Single intraperitoneal injections of imipramine and chlorpromazine were given in 100 μl of 0.9% NaCl at given intervals before the enzyme assays. Phentolamine and propranolol were given as repeated injections in 100 μl of 0.9% NaCl at 2 hr intervals beginning the injections 1 hr prior to the imipramine or chlorpromazine injections.

Determination of ODC and SAM-DC activities. For the enzyme assays the brain was homogenized with 3 vol of cold 25 mM Tris-HCl (pH 7.4 at 20°) containing 0.1 mM EDTA, 5 mM dithiothreitol and 0.1 mM pyridoxal 5′-phosphate. The homogenate was centrifuged at 105,000 *g* for 45 min at 4°. The assay conditions for ODC were essentially as described by Jänne and Williams-Ashman [24]. The reaction mixture contained 0.1 M Tris-HCl (pH 7.1 at 20°), 4 mM EDTA, 4 mM dithiothreitol, 0.4 mM pyridoxal 5′-phosphate, 0.42 mM [^{14}C]L-ornithine (2.27 mCi/mmol) and 0.19 ml of 105,000 *g* supernatant fraction in a total volume of 0.25 ml. Incubation was carried out in 10-ml glass tubes which were equipped with polypropylene center wells attached to rubber stoppers (Kontes Glass Co., Vineland, NJ). After 30-min incubation at 37°, the reaction was stopped by injecting 1 ml of 50% (w/v) trichloroacetic acid through the stopper. The $^{14}CO_2$ released from the medium was trapped in 0.3 ml of ethylene glycol/ethanolamine (1:1, v/v) which was mixed with 5 ml of Bray's scintillation fluid (25) for measurement of radioactivity.

The activity of SAM-DC was measured as outlined by Jänne and Williams-Ashman [26]. The assay mixture contained 0.1 M potassium phosphate (pH 7.4 at 20°), 2.5 mM putrescine, 0.16 mM *S*-adenosyl-L-[carboxyl-^{14}C]methionine (1.07 mCi/mmol), 6.7 mM dithiothreitol, 1 mM EDTA, and 0.1 ml of the 105,000 *g* supernatant fraction in a total volume of 0.15 ml. After a 30-min incubation at 37°, the samples were processed as described above for ODC measurement. Trichloroacetic acid was added to SAM-DC blanks before starting the reaction. ODC blanks contained 1 mM α-difluoromethylornithine, a specific irreversible inhibitor of the enzyme [27]. This enables the estimation of nonspecific decarboxylation of L-ornithine that has been pointed out using preparations of neural origin [28, 29].

Means in different experimental groups were compared using Student's *t*-test (two tailed, *t*-independent).

Chemicals. Unlabelled *S*-adenosyl-L-methionine was synthetized by the method originally described by Cantoni and Durrel [30], as modified by Pegg and Williams-Ashman [31]. L-[1-^{14}C]Ornithine (56 mCi/mmol) and *S*-adenosyl-L-[carboxyl-^{14}C]methionine were purchased from the Radiochemical Centre (Amersham, Bucks., United Kingdom). Phentolamine (Regitin®) was purchased from Ciba-Geigy Ltd. (Basle, Switzerland), propranolol from ICI Ltd. (Macclesfield, Cheshire, United Kingdom), chlorpromazine from Oy Star AB (Tampere, Finland) and imipramine from Lääke Oy (Turku, Finland). α-Difluoromethylornithine (RMI 71.782) was a generous gift from the Centre de Recherche Merrell International (Strasbourg, France). Other chemicals were either from Merck (Darmstadt, Germany) or Sigma (Saint Louis, MO) and were of the highest available purity grade.

RESULTS

Our preliminary experiments revealed that maximal responses in mouse cerebral ODC and SAM-DC activities occur 5 and 8 hr after imipramine and chlorpromazine injections, respectively. The dose responses summarized in Fig. 1 were assayed at these time points. The results show that maximal effect of chlorpromazine was reached at a dose of 50 mg/kg. Higher doses did not decrease significantly SAM-DC activity and, on the other hand, ODC activity started to decline with doses higher than that. Imipramine caused linear responses on both of the enzymes up to the dose of 120 mg/kg. Higher imipramine doses were not tested since 100 mg/kg on the dosage was lethal to some animals.

Based on the dose response studies the time courses of enzyme activities were measured up to 25 hr after injections of 50 mg/kg of chlorpromazine and 80 mg/kg of imipramine. Figure 2 shows that maximal ODC stimulations (5.5- and 7-fold) occurred 6 hr after imipramine and 8 hr after chlorpromazine injection, respectively. SAM-DC activity decreased simultaneously to 38 and 56% from the control level.

The imipramine- and chlorpromazine-induced increase in ODC activity was totally blocked by β-adrenoceptor blocker, propranolol, but not by α-adrenoceptor blocker, phentolamine, which in fact slightly potentiated the effect of imipramine on ODC (Table 1). However, neither of these blockers did abolish the inhibition of SAM-DC (Table 1). Both phentolamine and propranolol turned out to have a slight inhibitory effect on SAM-DC activity *in vivo*. When the possible *in vitro* effect of the drugs on ODC and SAM-DC activities was determined, an unexpected inhibition of SAM-DC activity by imipramine and chlorpromazine was observed (Table 2). None of the above drugs elicited a statistically significant changes in the cerebral ODC activity *in vitro* (data not shown). Experiments, which were conducted using different concentrations of imipramine and chlorpromazine, showed the inhibition of SAM-DC activity to be linear. Furthermore, the inhibition was not dependent on the concentration of substrate, *S*-adenosyl-L-methionine, or the activator, putrescine (data not shown).

DISCUSSION

The present results show that both chlorpromazine and imipramine elevate significantly mouse brain ODC activity. Due to the fact that this study was performed by using the whole brain and the doses of chlorpromazine and imipramine high enough to produce central effects other than their major effects

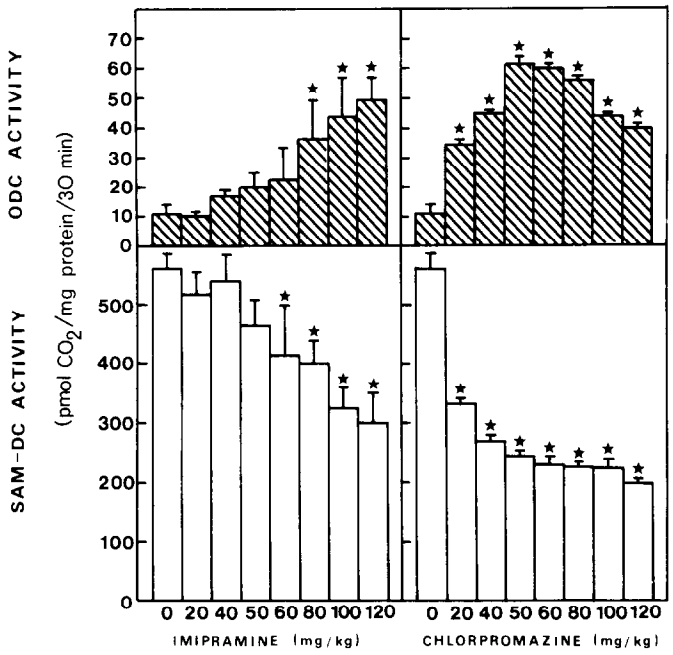

Fig. 1. Dose response of mouse brain ODC (▨) and SAM-DC (□) activities by imipramine and chlorpromazine treatment. Into the mice of NMRI strain were injected intraperitoneally single indicated doses of imipramine and chlorpromazine. Animals were killed 5 hr after imipramine and 8 hr after chlorpromazine injection. The bars show mean ± S.D. for at least six animals/group. The values significantly different from the control level are indicated by *asterisks* ($P < 0.001$).

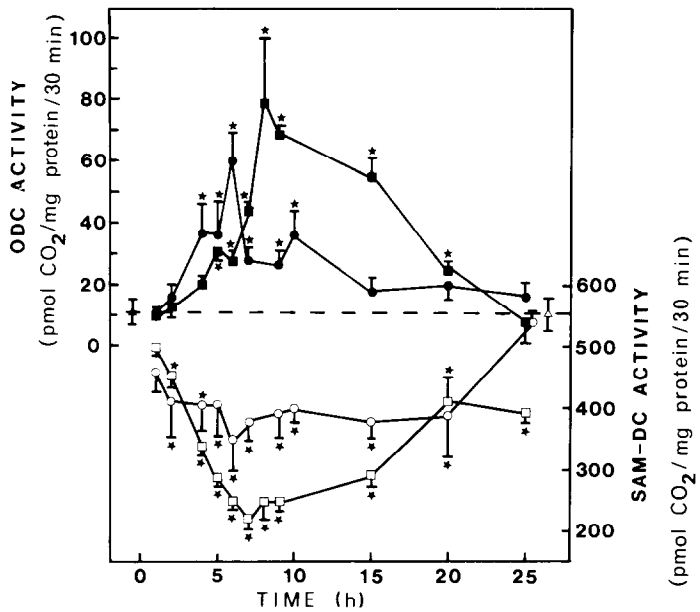

Fig. 2. Mouse brain ODC (closed symbols) and SAM-DC (open symbols) activities after imipramine (circles) and chlorpromazine (squares) injections. Groups of NMRI mice were treated with single doses of imipramine (80 mg/kg) and chlorpromazine (50 mg/kg) and killed at indicated time intervals. Each symbol with a vertical bar shows the mean ± S.D. for a given experimental group comprising at least six animals. *Asterisks* indicate significantly different ($P < 0.001$) values from the control level (11.32 ± 3.24 and 562.27 ± 28.25 pmol CO_2/mg protein/30 min for ODC and SAM-DC, respectively).

Table 1. The effect of combined administration of phentolamine or propranolol with imipramine and chlorpromazine on ODC and SAM-DC activities

Treatment	ODC activity		SAM-DC activity	
	pmol CO_2/mg protein/30 min			
		P (vs control)		P
Untreated	11.3 ± 3.2	—	562.3 ± 28.3	—
Phentolamine	19.7 ± 1.0	<0.001	502.5 ± 8.3	<0.001
Propranolol	15.5 ± 0.4	—	466.1 ± 0.3	<0.001
Imipramine	36.6 ± 13.6	<0.001	402.1 ± 44.3	<0.001
Chlorpromazine	79.1 ± 21.0	<0.001	247.5 ± 28.9	<0.001
		P (vs imipramine-treated)		P
Imipramine + phentolamine	87.9 ± 23.2	<0.001	388.0 ± 53.6	—
Imipramine + propranolol	11.2 ± 2.8	<0.01	276.2 ± 30.8	<0.001
		P (vs chlorpromazine-treated)		P
Chlorpromazine + phentolamine	116.5 ± 32.7	—	232.0 ± 27.9	—
Chlorpromazine + propranolol	4.1 ± 1.5	<0.001	247.0 ± 13.4	—

Mice of NMRI strain were treated with intraperitoneal injections of imipramine (80 mg/kg), chlorpromazine (50 mg/kg), phentolamine (10 mg/kg, every 2 hr) and propranolol (10 mg/kg, every 2 hr). The treatment with phentolamine and propranolol was started 1 hr before single dose of imipramine or chlorpromazine. Animals were killed 5 hr after imipramine and 8 hr after chlorpromazine injection.

[reviewed 32], the postulates about the correlation between the stimulation of ODC activity and neurochemical events would be pure speculation. However, the results suggest that the stimulation is dependent on β-adrenoceptors, since it can be abolished by a nonselective β-blocking agent, propranolol [33, 34], whereas phentolamine, a nonselective α-blocking agent [33, 34], was ineffective. Any firm conclusion, however, cannot be drawn, since imipramine and chlorpromazine are extensively metabolized drugs and propranolol is known to interfere with the metabolism of several compounds [35, 36], so that the antagonism by propranolol of the stimulation of ODC activity by imipramine or chlorpromazine might be due to an inhibition of the metabolism of these drugs by propranolol.

Table 2. Action *in vitro* of imipramine, chlorpromazine, phentolamine and propranolol on mouse brain SAM-DC activity

	Concentration (mM)	Relative activity of SAM-DC (%)
Control	—	100.0 ± 5.0 (12)
Imipramine	0.25	90.3 ± 8.1 (6)
	0.50	85.1 ± 5.3* (6)
	1.00	65.5 ± 9.8* (6)
	2.50	37.7 ± 7.8* (6)
	5.00	14.0 ± 9.0* (6)
Chlorpromazine	0.25	83.1 ± 4.2* (6)
	0.50	77.3 ± 3.8* (6)
	1.00	55.5 ± 1.0* (6)
	2.50	30.1 ± 5.2* (6)
	5.00	2.9 ± 0.5* (6)
Phentolamine	1.00	90.9 ± 12.1 (6)
Propranolol	1.00	100.8 ± 4.0 (6)

Relative activity ± S.D. of the numbers of determinations shown in brackets. Significance vs control: *P < 0.001. Experimental details for the enzyme assay are described in Materials and Methods.

In the studies on brain two types of SAM-DC responses have been demonstrated. In most cases the activity of SAM-DC behaves in the opposite manner than that of ODC, i.e. SAM-DC activity has its nadir at the time of ODC peak activity [e.g. 9–11]. However, in a few cases brain SAM-DC activity has been reported to increase concomitantly with that of ODC [37, 38]. No conclusions on the probable factors behind the different types of responses of SAM-DC activity can be made on the basis of existing knowledge. The present results show that the inhibition of SAM-DC in the case of imipramine, but not of chlorpromazine, is potentiated by propranolol. This may be due to the fact that propranolol is inhibitory to the enzyme *in vivo* even when given alone (Table 1), and this effect comes visible because of the relatively weak inhibition by imipramine. The clearly stronger inhibitory effect of chlorpromazine, on the other hand, may prevent seeing that of propranolol.

The comparison of the present results with any earlier studies is complicated by the quite unexpected finding that both imipramine and chlorpromazine caused the inhibition of SAM-DC also *in vitro*. The significance of the observed inhibition is emphasized by the fact that it occurs at concentrations which are low enough to justify the claim of possible physiological importance [see 39]. This suggests that the inhibition of SAM-DC activity is the initial change in the cerebral polyamine metabolism. The induction of ODC could then be a compensatory response, because its immediate product, putrescine, acts as an essential and specific activator of SAM-DC [40]. This possibility is emphasized by our recent observation (manuscript under preparation) that the inhibition of SAM-DC by i.c.v. injection of its specific inhibitor, methyl-glyoxal bis(guanylhydrazone)

[41], results in a significant stimulation of ODC activity and consequent accumulation of putrescine in the mouse brain. The inverse correlation of ODC and SAM-DC activities might be also explained by the observation on cultured mammary gland, where exogenous addition of putrescine results in the decrease in both the activity and the amount of SAM-DC [42]. In other words, the accumulation of putrescine by drugs decreases the activity of SAM-DC. However, this postulate can not explain the phenomenon that the ODC induction by imipramine and chlorpromazine can be totally blocked by propranolol without abolishing the inhibition of SAM-DC activity. This supports the former explanation and suggests that these two enzymes are independently regulated.

The mechanism of *in vitro* inhibition of SAM-DC activity observed in the present work remains to be established. Preliminary experiments that were carried out using the crude enzyme preparation described in Materials and Methods, suggest that the inhibition is noncompetitive in respect of both *S*-adenosyl-L-methionine and the activator, putrescine. However, the difficulties faced in determining the mode of action of methylglyoxal bis(guanylhydrazone) on SAM-DC [43, 44] emphasize that careful studies are required before any firm conclusions can be made.

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