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ACUTE AND SHORT-TERM EFFECTS OF LITHIUM ON GLUTAMATE METABOLISM IN RAT BRAIN*

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Abstract—Amino acids of the glutamate family, viz. glutamic acid, aspartic acid, glutamine, γ-aminobutyric acid (GABA) and alanine, along with the activities of glutamic acid dehydrogenase (GDH), aspartic acid aminotransferase (AST), alanine aminotransferase (ALT), glutamine synthetase (GS), glutaminase, glutamic acid decarboxylase (GAD) and GABA-aminotransferase (GABA-T) were estimated in cerebral cortex, cerebellum and brain stem of rats treated with a single dose of lithium or with seven daily doses of lithium (3 m-equiv./kg body wt). The levels of GABA were found to increase in cerebral cortex and brain stem following the administration of a single dose and also were found to be increased in cerebral cortex and cerebellum after treatment for 7 days. The content of glutamic acid was increased in all three brain regions after treatment for 7 days. Glutamine was increased in both cerebral cortex and brain stem after treatment for 7 days, whereas aspartic acid was increased in brain stem after both the administration of single dose and treatment for 7 days. A significant increase ($P < 0.05$) in the activity of GS was observed in brain stem after 7 days of treatment. Similarly, a significant increase ($P < 0.01$) in the activity of AST was observed in all three regions of the brain following the treatment for 7 days. The above results are discussed in relation to the known effects of lithium on brain cation metabolism and a suggestion is made that an imbalance in the functional activities of glutamic acid and GABA as a result of quantitative changes in these amino acids, brought about by lithium, may play a role in the therapeutic efficacy of lithium in bipolar disorders.

Administration of lithium salts in patients suffering from manic depressive psychoses has been reported to be therapeutically effective; the response in mania, however, is also reported to be occurring quickly within 5–10 days of treatment [1], thus indicating that lithium may exert acute effects on brain metabolism or may correct acutely the deranged metabolism of brain in mania. Derangement in the metabolism of biogenic amines is often implicated in affective disorders, and the therapeutic benefit of lithium has been attributed to its effects on the metabolism of monoamines [2, 3]. Lithium is also known to alter the electrolyte composition of various brain regions, particularly changing the content of sodium and potassium [3–6]. The actions of a number of neurotransmitters, such as biogenic amines, glutamic acid, γ-aminobutyric acid and glycine are terminated partly through uptake by synapses and surrounding glial cells, in addition to being degraded. Since reuptake of neurotransmitters is dependent on sodium, and since lithium is known to alter the metabolism of sodium, it is possible that lithium administration may result in changes in the metabolism of glutamic acid and GABA. Moreover, close interaction between amino acid and monoamine metabolism has been demonstrated in the brain (e.g. interaction between glutamatergic, GABAergic and dopaminergic pathways in basal ganglia). A derangement in the metabolism of monoamines may also result in a change in the metabolism of these two amino acids. Whereas glutamic acid is neuroexcitatory, GABA, which is derived from glutamic acid, acts as a neuro-inhibitory amino acid. The therapeutic benefit obtained by lithium in mania may occur partly as a result of its effect on the metabolism of glutamic acid and GABA. In this paper, we present the effects of lithium (after the administration of a single dose or after daily administration for 7 days) on brain region content of glutamic acid, glutamine, GABA, aspartic acid and alanine, along with its effects on the activities of glutamine synthetase (GS), glutaminase, glutamic acid decarboxylase (GAD), GABA aminotransferase (GABA-T), glutamic acid dehydrogenase (GDH), aspartic acid aminotransferase (AS) and alanine aminotransferase (ALT) in rat cerebral cortex, cerebellum and brain stem. Knowledge of the metabolism of the glutamate family of amino acids in various regions of the brain after administration of lithium for 7 days may throw light on the interaction of lithium with the metabolism of glutamic acid and GABA and may provide evidence for the mechanism of its beneficial effect in the treatment of mania.

MATERIALS AND METHODS

Animals. Albino rats belonging to a local strain with body weights of 150–200 g were used for the study.

Lithium treatment. To study the acute effects of lithium, lithium carbonate was administered intra-

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peritoneally at a dose of 3 m-equiv./kg body weight, and animals were killed 30 min after the injection by decapitation. To study the effects of repeated administration of lithium, daily intraperitoneal injections of lithium carbonate at a dose of 3 m-equiv./kg body weight were given for 7 days and animals were decapitated 30 min after the last injection. Animals had free access to food and water.

Preparation of homogenates. The brains were quickly removed from the heads. Cerebral cortex, cerebellum and brain stem were quickly separated according to the procedure of Sadasivudu and Lajtha [7]. The homogenates of each region were prepared in either 0.25 M sucrose or an appropriate buffer, using a Potter–Elvehjem type homogeniser with a teflon pestle.

For the estimation of amino acids, 50 mg of each region was homogenised in 80% ethanol. The alcohol homogenate was centrifuged at 8000 rpm at 5° for 10 min in a refrigerated centrifuge. The alcohol extract was evaporated to dryness under vacuum in a warm water bath. The residue was dissolved in 0.2 ml of distilled water, and the amino acids were separated by paper chromatography by multiple development and then quantitated as described by Sadasivudu *et al.* [8].

Enzyme estimation. Glutamic acid dehydrogenase was assayed by the method of Schmidt [9] as described by Sadasivudu *et al.* [8]. Glutamic acid decarboxylase and GABA-aminotransferase were estimated by the method of Sytinsky *et al.* [10] as described by Sadasivudu and Murthy [11]. Glutamine synthetase was estimated by the method of Rowe *et al.* [12] as described by Sadasivudu and Rao [13]. Glutaminase was estimated by the method of Goldstein [14] as described by Saleem and Sadasivudu [15]. Aspartic acid aminotransferase and alanine aminotransferase were assayed by the method of Karmen [16] as described by Rani *et al.* [17].

RESULTS

Although no significant changes in the content of glutamine in cerebellum and brain stem were observed after the administration of a single dose of lithium, a significant increase in the same was observed in cerebral cortex (Fig. 1). A significant increase was observed, however, in the content of glutamine in the cerebral cortex and brain stem after the administration of lithium for 7 days. Similarly, the content of glutamic acid was found to increase in all three brain regions only in rats treated with lithium for 7 days. A significant increase in the content of GABA was observed in cerebral cortex and brain stem after the administration of a single dose. The content of GABA was also found to increase significantly in cerebral cortex and cerebellum as a result of administration of lithium for 7 days. Aspartic acid was found to increase in brain stem in rats treated with a single dose and also for 7 days. The content of alanine was found to increase in cerebellum after the administration of a single dose and in cerebral cortex and brain stem after 7 days administration.

The activity of glutamine synthetase was found to show a significant increase only in brain stem in rats treated with lithium for 7 days (Fig. 2). Glutaminase was found to increase significantly only in cerebellum following the administration of a single dose. The

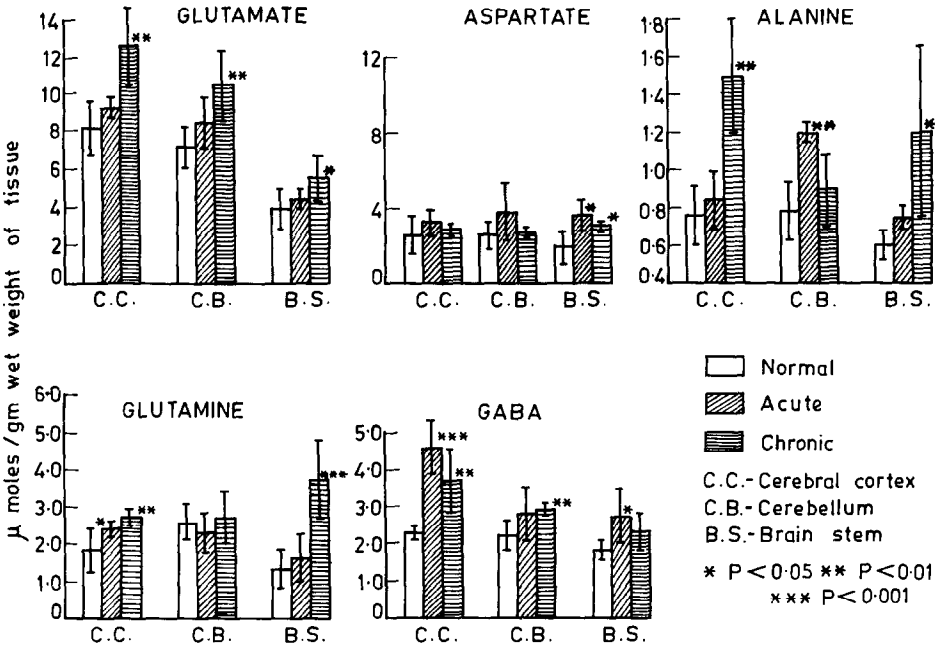

Fig. 1. Effect of lithium on amino acid content of different rat brain regions (mean ± S.D., N = 6). For methods see text. Acute: Administration of a single dose of 3 m-equiv. of lithium carbonate per kg body weight intraperitoneally; the animal was killed 30 min after the injection by decapitation. Short term: Administration of a single dose of 3 m-equiv. of lithium carbonate per kg body weight intraperitoneally daily for 7 days. The animal was decapitated 30 min after the last injection.

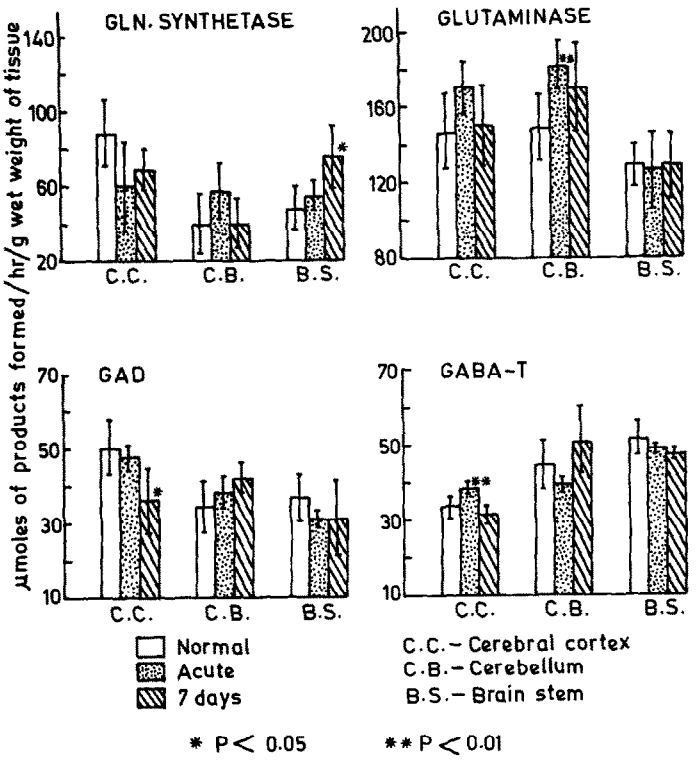

Fig. 2. Effect of lithium on activities of glutamine synthetase, glutaminase, GAD and GABA-T in different regions of rat brain (mean ± S.D., N = 6). See text for methods. Refer to the legend of Fig. 1 for treatment details.

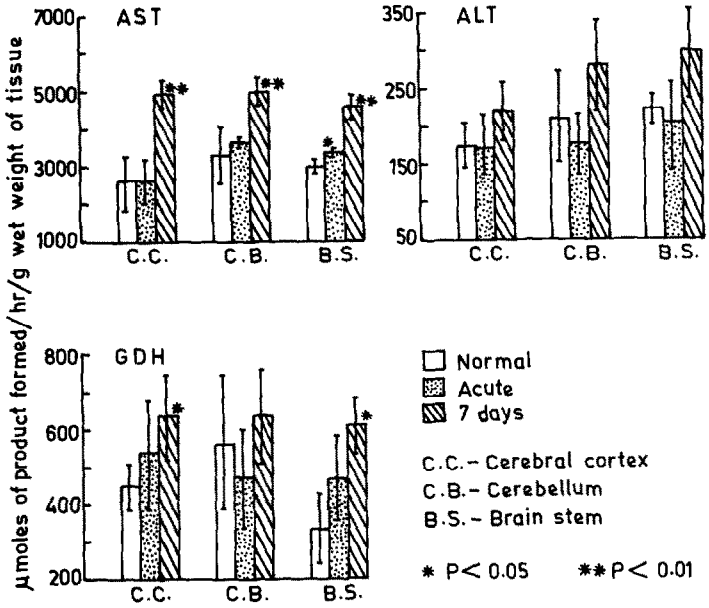

Fig. 3. Effect of lithium on the activities of AST, ALT and GDH in different regions of rat brain (mean ± S.D., N = 6). See text for methods. Refer to the legend of Fig. 1 for treatment details.

activity of GAD was found to decrease in cerebral cortex in rats treated with lithium for 7 days. A significant increase in GABA-T was observed in cerebral cortex following the administration of a single dose.

A significant increase in GDH was observed in cerebral cortex and brain stem following the administration of lithium for 7 days (Fig. 3). The activity of AST was found to increase in all three brain regions as a result of treatment with lithium for 7 days and in brain stem after the administration of a single dose. No significant change was observed in the activity of ALT in any of the three regions after either a single dose or seven daily injections.

DISCUSSION

It is fairly well established that a major portion of synaptic transmission in the central nervous system is mediated by neuroactive amino acids such as glutamic acid, aspartic acid, GABA and glycine [18]. While the former two act as neuro-excitatory amino acids, the latter two act as neuro-inhibitory amino acids. As suggested by Berl and Clarke [19], it is possible that in affective disorders associated with aggressive excitatory behaviour (mania) or with depressive behaviour (depression) or both (manic depressive psychosis), an imbalance in the functional activities of two groups of amino acids in certain parts of the central nervous system may occur.

Administration of lithium for 5–10 days is known to be effective in the treatment of mania, indicating that such beneficial effects are of an acute nature. Changes in glutamic acid content in mouse brain have been observed 20 min after intraperitoneal administration of a low dose (0.25 to 0.5 mmole per mouse) by DeFeudis and Delgado [20]. In the present study higher doses of lithium (0.45 to 0.6 mmole per rat) were administered and, as such, the effect must have been clearly due to lithium administration, although it is not known whether sufficient lithium penetrates into the brain with the dose employed. However, serum levels were reported to reach peak levels within 15–60 min after administration. Moreover, the effects of lithium on brain have been reported to correlate better with serum levels than with brain levels [19]. The observed significant increase in the content of GABA in cerebral cortex and brain stem after a single dose of lithium might result in a state of inhibition in these two brain regions and may explain the therapeutic benefit of lithium in overcoming mania. Pharmacological agents which act as GABAergic agonists (valproic acid) have been reported to be useful in affective disorders [21, 22]. The content of GABA remained elevated in cerebral cortex and cerebellum even after seven daily doses. Although the exact mechanism involved in the rise in GABA levels is not clearly understood, such changes in the content of GABA might tilt the balance of synaptic activity in favour of inhibition. However, the significant increase in the content of glutamic acid in cerebral cortex and cerebellum under these experimental conditions (after 7 days) indicates that any acute inhibitory effects brought about by lithium slowly diminished, giving place to a state of excitation. Such a dichotomy in the pharmacological effects of lithium may be one of the factors in its therapeutic efficacy in the treatment of bipolar disorders.

Lithium is known to disturb the metabolism of Na^+ and K^+ in brain giving rise to a greater efflux of Na^+ from cells, probably through its effect on Na^+, K^+-activated ATPase [23, 24]. Consequently, the reuptake of the released glutamic acid or GABA at their respective nerve endings and also by surrounding glial cells may be diminished. This may be one of the factors responsible for the observed increase in the content of GABA and glutamic acid. Increased content of GABA in CSF has been reported in patients receiving lithium for treatment of bipolar disorders [25]. However, increased production of these amino acids may also occur after administration of lithium. Increased transfer of glucose and its utilisation in brain [26] followed by increased labeling of glutamate from uniformly labeled [^{14}C]glucose has been reported [27]. Such an increase in glutamic acid may give rise to more GABA in cerebral cortex, although no significant change in GAD activity was observed under these conditions. Although no significant change in the activity of GAD was observed in cerebellum, a significant increase in the activity of glutaminase, which is mostly localised in nerve endings, after the administration of lithium may suggest an increased conversion of glutamine to glutamic acid to be converted to GABA in GABAergic nerve endings. The observed decrease in GAD activity in cerebral cortex after 7 days of lithium administration may be regarded as compensatory and would trigger a reduction in the process of inhibitory activity which would be helpful in controlling depression, being the next phase following mania in the cyclical bipolar disorder. The increase of glutamine in brain stem after the administration of lithium for 7 days could be attributed to the observed increase in the activity of glutamine synthetase in this region. Glutamine synthetase is exclusively localised in glial cells [28] and is involved in the metabolism of a more active smaller pool of glutamic acid in the brain [29, 30]. Although the normal glial uptake of glutamate, released from nerve endings, may be inhibited by lithium, the observed increase in GDH activity in brain stem under these conditions may facilitate the formation of more glutamic acid in glial cells for conversion to more glutamine. This would facilitate the removal of NH_3, a freely diffusible substance released from nerve endings by glutaminase as a result of spontaneous activity. Hence, following the administration of lithium, the synthesis of glutamine in glial cells may be facilitated in brain stem, from locally formed glutamic acid in glia rather than the glutamic acid released from nerve endings. The presence of GDH in glial cells has been well documented [31, 32]. Glutamine, so formed, may diffuse into the nerve endings, completing the latter half of the glutamine cycle between the nerve endings and surrounding glial cells [33]. The increase in glutamic acid in brain stem under these conditions may be due to the accumulation of released glutamic acid from glutamine. Brain stem is the seat of the ascending reticular activating system. Accumulation of glutamic acid may activate this pathway under these

circumstances and may help to ward off any depression that may set in.

The increased activities of AST in all three brain regions following the administration of lithium for 7 days would also facilitate the formation of glutamic acid under these conditions. However, the reasons for the observed rise in aspartic acid and alanine in some regions of brain are not known. Administration of lithium for longer periods was found to bring about an increase in the content of certain amino acids (glycine, alanine, GABA, valine and lysine) in certain brain regions [3].

The mechanism involved in the observed changes in the activities of some of the enzymes belonging to the glutamic acid family following the administration of lithium is not known. *In vitro* addition of appropriate amounts of lithium to the homogenates of the normal brain regions was found to decrease the activity of glutamine synthetase, while no significant change was observed in the activities of other enzymes studied (not presented). This is contrary to at least one *in vivo* observation made in brain stem in the present study with respect to the activities of glutamine synthetase which showed an increase. Obviously, the inhibition of glutamine synthetase may be attributed to the competitive interference of lithium with Mg^{2+} required for glutamine synthetase activity. Lithium is known to exert such an interference on Mg^{2+}-dependent enzyme reactions [34–36]. The observed increase in glutamine synthetase activity in the brain stem and the absence of an inhibition in the activity of this enzyme, under these conditions, may therefore be due to local metabolic changes overcoming the inhibitory effects of lithium on this enzyme.

In conclusion, the bizarre and differential changes in the content of glutamic acid, GABA, glutamine, aspartic acid and alanine along with the changes in the activities of associated enzymes in the different regions following the administration of lithium may be mostly attributed to a probable disruption in the well known compartmentalised metabolism of these amino acids in normal brain. This disturbance may be due to the effects of lithium on the metabolism of brain cations such as Na^+, K^+, Mg^{2+} and Ca^{2+}. Consequently, the release and the uptake of neurotransmitter amino acid may be affected and, as such, the levels of these amino acids may not correspond exactly to the activities of the various enzymes involved in their synthesis and degradation. Probably such an effect of lithium may be one of the factors in the efficacy of treatment of bipolar disorders by lithium.

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