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Brain Serotonin Metabolism with Relation to the Head Twitches Elicited by Lithium in Combination with Reserpine in Mice

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FURUKAWA, T , K YAMADA, Y KOHNO AND N NAGASAKI *Brain serotonin metabolism with relation to the head twitches elicited by lithium in combination with reserpine in mice* PHARMAC BIOCHEM BEHAV **10**(4) 547–549, 1979 —Lithium alone, which scarcely induced head twitches, did not affect brain 5-hydroxytryptamine (5-HT) levels but increased 5-hydroxyindoleacetic acid (5-HIAA) levels However, the combined use of lithium chloride (2 mEq/kg×5, SC) and reserpine (5 mg/kg, SC) administered hourly markedly elicited the head twitches, together with the pronounced decrease of 5-HT levels and increase of 5-HIAA levels as similarly seen by reserpine alone Pretreatment with p-chlorophenylalanine (PCPA) strongly potentiated the twitches elicited by the combined administration of lithium and reserpine, along with inducing the significant decrease of 5-HT levels and no change of 5-HIAA levels as compared with those levels in the PCPA-treated mice The results imply that lithium produces the head twitches in the presence of reserpine, and an increase of 5-HIAA or a decrease of 5-HT levels do not necessarily interfere with the incidence of the twitches, and that the receptor sensitivity to 5-HT is strongly involved in the incidence of the head twitches

Lithium Reserpine Head twitches Serotonergic neurons 5-HT 5-HIAA

IT HAS been proposed that the serotonergic neurons are involved in head twitches, since large doses of 5-hydroxytryptophan (5-HTP) induce head twitches in mice, concomitant with an increase in formation of brain 5-hydroxytryptamine (5-HT) [4, 7, 9, 12] However, in spite of the fact that both tricyclic antidepressants and monoamine oxidase inhibitors cause a further increase of the brain 5-HT level in the 5-HTP-treated animals [2,10], the former drugs inhibit the 5-HTP-induced head twitches whereas the latter potentiate this behavioral pattern [4,7] Recently, it was reported that the 5-HTP-induced head twitches were potentiated by an increase of 5-HT level and reduced by an increase of 5-hydroxyindoleacetic acid (5-HIAA) level or a decrease of 5-HT level in the brain of mice using isocarboxazid, amitriptyline and probenecid [10] Therefore, the serotonergic mechanism involved in the head twitches may be more complex. The present authors recently found that lithium or rauwolfia alkaloids alone did not elicit twitching but the combined administration of both drugs did elicit the twitches in mice [15]

The present investigation was performed in an attempt to understand the serotonergic mechanisms involved in the combined use of lithium and reserpine-induced head twitches by determining brain 5-HT and 5-HIAA levels.

METHOD

Animals

Animals in the experiment were healthy ddY male albino mice obtained from Kyudo Animal Laboratory (Kumamoto, Japan) They were caged in groups of 10 for a week before as well as throughout the experiment. The body weights of mice were 22–26 g at the beginning of experiment The food consisted of MF, Oriental Yeast Ltd The animals were permitted food and water ad lib except during the experimental trials. All trials and breeding were carried out at an environmental temperature of 24 ± 1°C and a humidity of 50 ± 10%

Procedure

Measurement of head twitches Head twitches are proposed to be spontaneous irregularly occurring head-shakes, which resemble a strong pinna reflex involving the whole head of the animal but occur without tactile stimulation un-

like the pinna reflex or a slow side-to-side movements of the head [4] A group of 5 mice were placed in a plastic box (33×25×11 cm), and the number of this type of head twitch was counted for a 10-min period 2 hr after injection of reserpine or saline as control

Assaying the endogenous amine The mice were killed by decapitation 4 hr after the last injection of lithium, 2 hr after reserpine or 9 hr after the last injection of PCPA The brain was rapidly removed and, after removing the cerebellum, the brain tissue was frozen at −20°C and stored until homogenization Simultaneous determination of 5-HT and 5-HIAA was done in each brain by the method described by Curzon and Green [6] with a modification [8], and fluorescence intensity was determined by a spectrofluorophotometer (Simadzu RF 501)

Administration of drugs The mice were treated with a 5 times hourly injection of saline as a control or of lithium chloride (2 mEq/kg, SC) Reserpine (5 mg/kg, SC) was administered 2 hr after the last injection of lithium. PCPA (300 mg/kg×3, PO) was administered 49 hr, 25 hr and 1 hr before the first injection of lithium

Drugs

The drugs used were lithium chloride (Kishida Chemicals), reserpine (Apoplone, Daiichi Pharmaceutical), dl-p-chlorophenylalanine (Nakarai Chemicals) and carboxymethyl cellulose (Ishizu Pharmaceutical) Lithium chloride was dissolved with distilled water, sterilized and adminsterred subcutaneously using a 0 5 ml syringe in a volume of 0 1 ml/10 g PCPA was administered as a suspension in 0 25% carboxymethyl cellulose

Statistical Analysis

The number of head twitches and tissue amine concentrations were expressed as mean values ± standard error of the mean, and statistical analysis was calculated using the two-tailed Student's *t*-test

RESULTS

Head Twitches Induced by Lithium Administered in Combination with Reserpine

As shown in Table 1, 5 times hourly administration of lithium (2 mEq/kg×5, SC) or single injection of reserpine (5 mg/kg, SC) exhibited occasional head twitches in a small proportion of mice (0 3–1 3), while the marked head twitches (8 1 ± 1 4) were elicited by the combined treatment with lithium and reserpine The twitches were markedly increased (18 0 ± 3 9) by treatment with PCPA (300 mg/kg×3, PO) before lithium and reserpine

Effects of Lithium in Combination with Reserpine on Brain 5-HT and 5-HIAA Contents

As shown in Fig 1, the control levels of brain 5-HT and 5-HIAA obtained from the saline-treated mice (n=16) were 406 ± 10 ng/g and 351 ± 14 ng/g, respectively Lithium alone did not affect 5-HT levels but increased 5-HIAA levels Administration of reserpine exhibited a marked decrease of 5-HT levels and increase of 5-HIAA levels The combined administration of lithium and reserpine also decreased brain 5-HT levels to 48% and increased 5-HIAA levels to 248% in comparison with the control levels After injection of PCPA, brain 5-HT and 5-HIAA levels were mark-

TABLE 1

NUMBER OF HEAD TWITCHES AFTER ADMINISTRATION OF DRUGS

Drugs	N	Head twitches (mean ± SE)
Saline	10	0 6 ± 0 3
Li 2 mEq/kg × 5	10	1 3 ± 0 8
Reserpine 5 mg/kg	10	0 3 ± 0 2
Li 2 mEq/kg × 5 + Reserpine 5 mg/kg	20	8 1 ± 1 4*
PCPA 300 mg/kg × 3 + Li 2 mEq/kg × 5 + Reserpine 5 mg/kg	10	18 0 ± 3 9*†

Mice were treated with a 5 times hourly injection of saline as a control or of lithium chloride (Li) Reserpine was administered 2 hr after the last injection of lithium p-Chlorophenylalanine (PCPA) was administered 49 hr, 25 hr and 1 hr before the first injection of lithium The number of head twitches was counted for a 10-min period 2 hr after injection of reserpine or saline as control *Significantly different from the saline-treated group, $p<0$ 01 (two-tailed Student's *t*-test)

†Significantly different from the lithium plus reserpine-treated group, $p<0$ 01

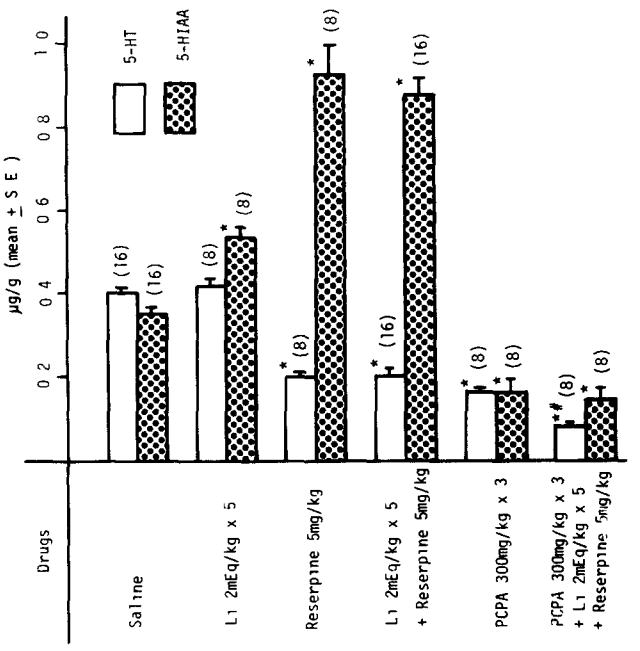

FIG 1 Effects of lithium in combination with reserpine on brain 5-HT and 5-HIAA contents in the non-pretreated and PCPA-pretreated mice Animals were killed by decapitation 4 hr after the last injection of lithium (Li), 2 hr after reserpine or 9 hr after the last injection of p-chlorophenylalanine (PCPA) Number in parenthesis indicates number of mice used *Significantly different from the saline-treated group, $p<0$ 01 #Significantly different from the PCPA-treated group, $p<0$ 01

edly decreased to 39% and to 46%, respectively, from the control levels The combined administration of lithium and reserpine in the PCPA-treated mice induced a significant decrease of 20% in brain 5-HT levels and no change in 5-HIAA levels as compared with those levels obtained from the PCPA-treated mice

DISCUSSION

Shaw and Ratcliffe [14] have proposed that lithium increases turnover of 5-HT through increases in release, reuptake and intraneuronal breakdown After treatment with lithium in this study, brain 5-HT levels were not altered but 5-HIAA levels were increased Therefore, lithium seems to facilitate a release and subsequent turnover of 5-HT Segawa and Nakano [13] also reported that 3 times hourly administration of lithium did not inhibit but twice daily administration of lithium for 4 days did inhibit the reserpine-induced release of 5-HT in rats by interfering with the releasing mechanism of reserpine at the synaptic vesicle Administration of lithium in combination with reserpine exhibited herein a marked decrease of brain 5-HT levels and increase of 5-HIAA levels as similarly seen by reserpine alone Accordingly, the repeated injections of lithium in one day did not reduce the depletion rate of brain 5-HT and the increase rate of brain 5-HIAA elicited by reserpine The 5-HTP-induced head twitches were inhibited by tricyclic antidepressants, which induced an increase of both 5-HT and 5-HIAA levels, and by probenecid, which caused an increase of 5-HIAA levels without affecting 5-HT levels in the brains of 5-HTP-treated mice [10] There has been, thus, the proposal that the increase of 5-HIAA or decrease of 5-HT inhibits the head twitches [10] However, in the present studies, the remarkable reserpine-produced increase of 5-HIAA levels and decrease of 5-HT levels did not prevent an incidence of head twitches elicited by lithium in combination with reserpine

Meanwhile, it was reported that tricyclic antidepressants inhibited the uptake of tryptophan [2] and 5-HT [3], decreased the 5-HT depletion after synthesis inhibition [2,5], and retarded the disappearance of labeled 5-HT from the brain [11], suggesting a decrease of brain 5-HT turnover caused by the drugs Therefore, a decreasing action of tricyclic antidepressants in brain 5-HT turnover seems to be a possible mechanism involved in the inhibitory effect of this drug on the 5-HTP-induced head twitches.

It was proposed that destruction of the central serotonergic neurons by treatment with 5,7-dihydroxytryptamine resulted in producing the postsynaptic supersensitivity [1] Administration of PCPA revealed a marked decrease of brain 5-HT levels Therefore it can be assumed that compensatory mechanisms, i e , postsynaptic supersensitivity to 5-HT, develops as a consequence of a depletion of 5-HT after treatment with PCPA In addition, as suggested herein by changes in 5-HT and 5-HIAA levels, the combined administration of lithium and reserpine seems to induce a release of residual 5-HT even in the PCPA-pretreated mice but to a smaller extent than that observed in the PCPA-untreated mice Accordingly, the postsynaptic supersensitivity of 5-HT receptors, which seems to be activated by newly synthesized and released 5-HT by lithium in combination with reserpine, may account for the potentiation in the head twitches observed by pretreatment with PCPA, a 5-HT synthesis inhibitor

These results imply that an increase of 5-HIAA or a decrease of 5-HT levels does not necessarily interfere with an incidence of head twitches and that postsynaptic serotonergic receptor activity plays an important role in the appearance of this behavioral pattern

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