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Suppressive Effects of Intraventricular Injected Dopamine and Nomifensine on Muricide Induced by Thiamine Deficiency

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ABE, Y., T. TADANO, A. YONEZAWA AND K. KISARA. *Suppressive effects of intraventricular injected dopamine and nomifensine on muricide induced by thiamine deficiency.* PHARMACOL BIOCHEM BEHAV **26**(1) 77–81, 1987.— The effects of dopamine (DA) and nomifensine (NF) on muricide activity induced by thiamine deficiency were examined. The chronic administration of L-dopa and nomifensine during feeding of thiamine deficient diet attenuated the muricide activity. Moreover, acute administration of L-dopa or nomifensine (IP) and dopamine or nomifensine (ICV) suppressed the thiamine deficiency-induced muricide activity dose-dependently. Small doses of apomorphine inhibited the muricide response significantly. The suppressive effects of dopamine and nomifensine were antagonized by pretreatment with 6-hydroxydopamine, but were not changed by pretreatment with p-chlorophenylalanine. These results suggest that the dopaminergic system has an important role in the regulation to the thiamine deficiency-induced muricide response.

Muricide Thiamine deficiency Dopamine Nomifensine Rats

WE have previously reported that thiamine deficiency causes muricide (mouse killing) response in rats [12]. Once the muricide response has appeared, it remains throughout the experimental periods, and can not be suppressed by an injection of thiamine hydrochloride and/or change to a complete diet [11]. Moreover, we have reported that thiamine deficient rats do not kill rat pups [12]. It has been reported that intraperitoneal injection of 5-hydroxytryptophan (5-HTP) and intraventricular injection of serotonin (5-HT) suppress this muricide response, and this suppressive effect of 5-HTP is augmented by the pretreatment with Ro4-4602, a peripheral dopa decarboxylase inhibitor [10]. These findings suggest that the suppression of the muricide response may involve central 5-HT neurons. On the other hand, the muricide response in olfactory bulb lesioned rat is suppressed by desipramine which is known to block noradrenaline (NA) reuptake activity at synapses, and by microinjection of NA [16]. The muricide response induced by tetrahydrocannabinol is suppressed by nomifensine (NF) which is known to have potent dopamine (DA) reuptake activity [1]. However, it remains unknown whether the suppressive effect of muricide is mediated by the central catecholaminergic system. The present experiment was therefore designed to investigate the effect of systemic administration of L-dopa and NF, and administration of ICV DA and NF.

METHOD

Animals

Male Wistar rats, weighing 70–80 g at the beginning of the feeding were obtained from Shizuoka Farm Co. DDY-male mice weighing 18–22 g were sacrificed for the muricide test. The animals were kept at constant temperature (22±1°C) with a constant relative humidity and the light cycle was automatically controlled (7:30–19:30 hr). The rats were housed individually in wire mesh cages (17×25×37 cm), and divided into the following two dietary treatment groups: (1) The thiamine deficient group was provided with a thiamine deficient diet and water ad lib. The thiamine deficient diet (Nihon CLEA Co.) consisted of a basic ration, including 67.6% carbohydrate, 18% lipid and supplemented with vitamins (except thiamine) and minerals. (2) The control group was supplied ad lib with the same complete diet, which was identical to the thiamine deficient group diet except that it contained 0.5 mg of thiamine hydrochloride per 100 g of diet. Statistical significance among the group was assessed by χ^2-test.

Muricidal Observation

The muricidal test was conducted for a 5 min period. One

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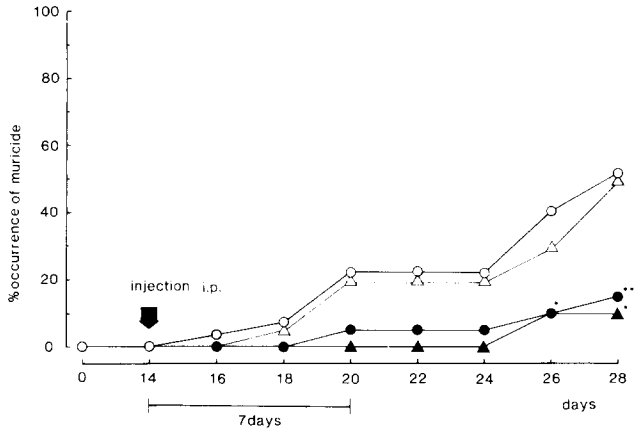

FIG. 1. The effect of chronic (7 days, once a day) treatment with saline IP (○) or nomifensine in a dose of 10 mg/kg IP (●) or L-dopa in a dose of 15 mg/kg IP (△) or 30 mg/kg IP (▲) on the incidence of muricide induced by thiamine deficiency. Number of animals per each group: 20–27. *$p<0.05$ when compared with saline treated group. **$p<0.01$ when compared with saline treated group.

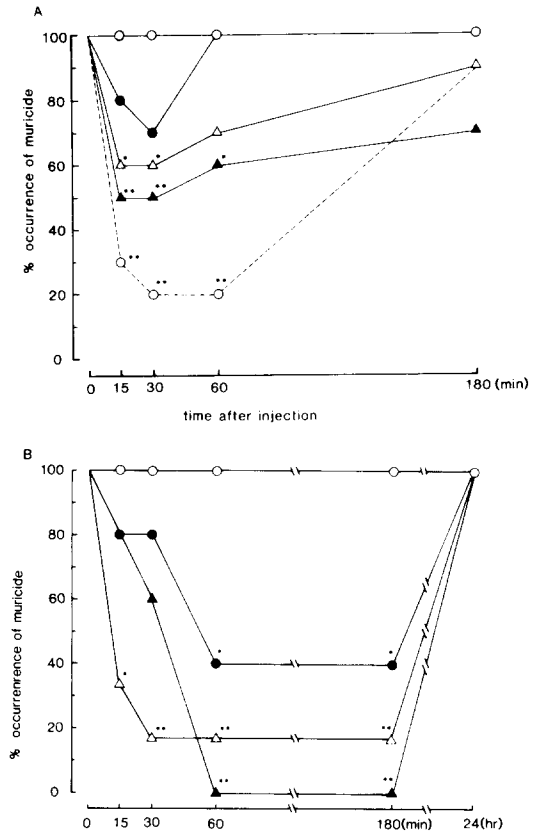

FIG. 2. A: The effect of intraperitoneal treatment with saline (○) or L-dopa in a dose of 15 mg/kg (●), 22.5 mg/kg (△) or 30 mg/kg (▲) on the muricide induced by thiamine deficiency. Ro4-4602 (5 mg/kg, IP) was pretreated 30 min before L-dopa 30 mg/kg, IP (○ with dashed line). Number of animals per each group: 10. *$p<0.05$ when compared with saline treated group. **$p<0.01$ when compared with saline treated group. B: The effect of intraventricular saline (○) or dopamine in a dose of 2.5 μg/rat (●), 10 μg/rat (△) or 40 μg/rat (▲) on muricide of thiamine deficiency. Number of animals per each group: 6. *$p<0.05$ when compared with saline treated group. **$p<0.01$ when compared with saline treated group.

hr before drug administration, the thiamine deficient rat was tested for its tendency to kill during a 5 min observation period. A mouse was introduced into the homecage. If the rat killed a mouse during this period, the rat was labeled as a killer-rat, and if it failed to kill a mouse during the 5 min observation period it was labelled as a non-killer-rat. The killer-rats were selected at random and pharmacological tests were carried out. The mice were removed at the end of the observation period, and if they were alive the killing response was termed as suppressed.

Drugs

The drugs used in this study were: L-3,4-hydroxyphenylalanine (L-dopa) (NAKARAI), dopamine (DA) hydrochloride (WAKO), 6-hydroxydopamine (6-OHDA) (SIGMA), Ro4-4602 (ROCHE), desmethylimipramine (CIBA-GEIGY), apomorphine hydrochloride (SIGMA) and nomifensine (NF) (HOECHST Japan). L-dopa was suspended in pyrogen-free saline containing 0.3% carboxymethyl cellulose. DA and 6-OHDA were dissolved in pyrogen-free saline solution containing to 0.1% ascorbic acid. All other drugs except L-dopa, DA and 6-OHDA were dissolved in pyrogen-free saline. For IP drug administration a volume of 0.2 ml/100 g body weight was used.

Intraventricular Injection of 6-OHDA, DA and NF

After 28 days of feeding a thiamine deficient diet, the thiamine deficient killer-rats were provided the complete diet (containing thiamine) (Nihon CLEA Co.) and water ad lib. About 7 days after giving the control diet, the rats still showed the muricide aggression. Rats weighing 180–220 g were selected for surgery. Each animal was anesthetized with sodium pentobarbital (30 mg/kg, IP) and a cannula which was a slightly modified metal hyperdermic needle (0.5 mm dia.), was implanted into the right ventricle of each rat according to the brain atlas of Pellegrino *et al.* [12]. At least 7 days were allowed for recovery from the surgical procedures before the experiment was begun. DA, 6-OHDA and NF were given intraventricularly (ICV) to the animals in a volume of 10 μl per animal with a graduated Hamilton microsyringe. When administration of 6-OHDA and desipramine (DMI) was combined, DMI (20 mg/kg) was injected IP on day 7 following ICV injection of 6-OHDA. On completion of the studies the location of the injection cannula and point of drug deposition were determined histologically.

RESULTS

Effect of 7 Successive Days of IP Administration of L-Dopa and NF

The incidence of the muricide response was gradually increased with days after thiamine deficient feeding (control group). When L-dopa and NF were administered IP once a day (9:00 a.m.) for 7 days from the 14th day to the 20th day during deficient feeding, the occurrence of the muricide activity was significantly suppressed when compared with

TABLE 1

EFFECT OF APOMORPHINE ON THE MURICIDE INDUCED BY THIAMINE DEFICIENCY

Treatment	Dose (mg/kg)	% Animal Exhibiting Suppression
Saline	0.1 ml/kg	0% (0/6)
Apomorphine	0.025 mg/kg	16.7% (1/6)
	0.05 mg/kg	50% (4/8)*
	0.1 mg/kg	25% (2/8)
	1.0 mg/kg	50% (4/8)*
	2.0 mg/kg	71.4% (5/7)†

Muricidal test was observed 30 min after the intraperitoneal administration of apomorphine.

*$p<0.05$, †$p<0.01$; when compared with saline control group.

saline-treated control group (L-dopa 30 mg/kg treated group, $\chi^2=3.147, p<0.05$ and NF 10 mg/kg treated group, $\chi^2=5.635$, $p<0.05$). Even after the administration of L-dopa and NF was stopped, this suppressive effect continued; the incidence of muricide activity on the 14th day after administration of L-dopa and NF (28th day after deficient feeding) was 10% and 15% respectively ($\chi^2=5.303, p<0.05$ and $\chi^2=6.758$, $p<0.01$) (Fig. 1).

Effect of IP Administration of L-Dopa and Apomorphine, and ICV Administration of DA

Intraperitoneal administration of L-dopa in doses of 15, 22.5 and 30 mg/kg produced a dose-dependent inhibition of the muricide response (Fig. 2A). At the time of peak effect, 30 min after IP administration of L-dopa, the incidence of muricide activity was 70% (15 mg/kg), 60% (22.5 mg/kg) and 50% (30 mg/kg). The ED50 for muricidal suppression by L-dopa IP was approximately 29 mg/kg (95% confidence limits; 14.8–56.8 mg/kg). Moreover, the suppressive effect of L-dopa was markedly augmented by concominant administration of Ro4-4602 (5 mg/kg, IP). The incidence of muricide activity at the time of peak effect was 50% for the L-dopa (30 mg/kg) treated group and 20% for the Ro4-4602 + L-dopa treated group (Fig. 2A). Almost complete recovery of the muricide activity was seen 3 hr after administration (Fig. 2A).

Intracerebroventricular administration of 2.5, 10 and 40 μg/rat of DA produced a dose-dependent inhibition of this muricide activity. The peak effect was obtained 30–180 min after the injection. At the time of peak effect, the incidence of muricide was 40% at 2.5 μg, 16.7% at 10 μg and 0% at 40 μg. Complete recovery of muricide was seen 24 hr after ICV administration of DA (Fig. 2B). The ED50 for muricidal suppression of DA ICV was 11.5 μg (95% confidence limits 4.1–31.9 μg/rat). Intraperitoneal administration of apomorphine in doses of 0.05, 1.0 and 2.0 mg/kg also suppressed muricide activity (Table 1).

Effect of IP and/or ICV Administration of NF

Intraperitoneal administration of 2.5, 5 and 10 mg/kg of NF produced a dose-dependent inhibition of the muricide. At the time of peak effect, 15–60 min after administration,

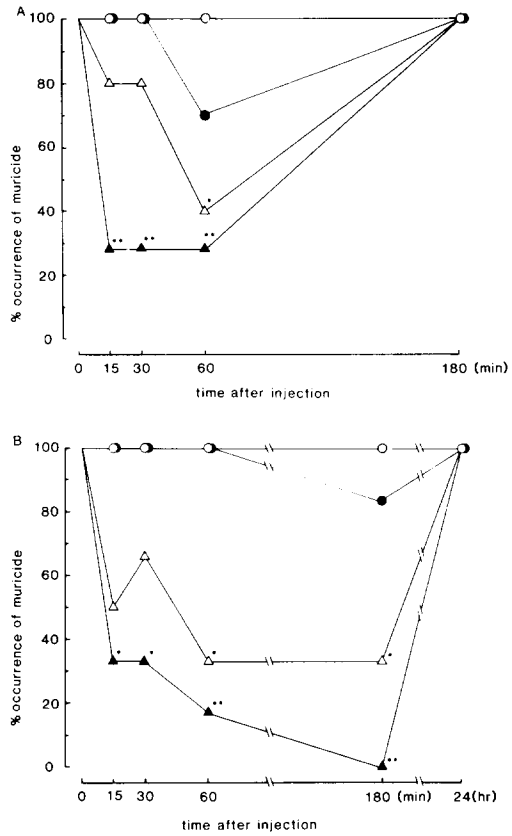

FIG. 3. A: The effect of intraperitoneal saline (○) or nomifensine in a dose of 2.5 mg/kg (●), 5 mg/kg (△) or 10 mg/kg (▲) on the muricide of thiamine deficiency. Number of animals per each group: 7–10. *$p<0.05$ when compared with saline treated group. **$p<0.01$ when compared with saline treated group. B: The effect of intraventricular saline (○) or nomifensine in a dose of 5 μg/rat (●), 7.5 μg/rat (△) or 10 μg/rat (▲) on muricide induced by thiamine deficiency. Number of animals per each group: 6. *$p<0.05$ when compared with saline treated group. **$p<0.01$ when compared with saline treated group.

the incidence of the muricide was 71.4% following a dose of 2.5 mg/kg, 40% following a dose of 5 mg/kg and 28.6% following the dose of 10 mg/kg. Complete recovery of muricide was seen 180 min after IP administration of NF (Fig. 3A). The ED50 for muricidal suppression of NF was approximately 6.6 mg/kg (95% confidence limits 5.1–8.6 mg/kg). Intracerebroventricular administration of NF (5, 7.5 and 10 μg/rat) produced a dose-dependent inhibition of the muricide activity. The peak effect was obtained 60–180 min after the injection. At the time of peak effect, the incidence of muricide was 83.3% at a dose of 5 μg/rat, 33.3% at a dose of 7.5 μg/rat and 0% at a dose of 10 μg/rat (Fig. 3B). ED50 for the muricidal suppression was 7.3 μg/rat (95% confidence limits; 6.6–8.1 μg).

Effect of 6-OHDA on the Suppressive Effect of DA and NF

As shown Fig. 2B, DA markedly suppressed the muricide aggression induced by thiamine deficiency. This suppressive effect of DA was antagonized by pretreatment with 6-OHDA + DMI (Fig. 4A). Pretreatment with 6-OHDA + DMI also antagonized the suppressive effect of NF (Fig. 4B) whereas

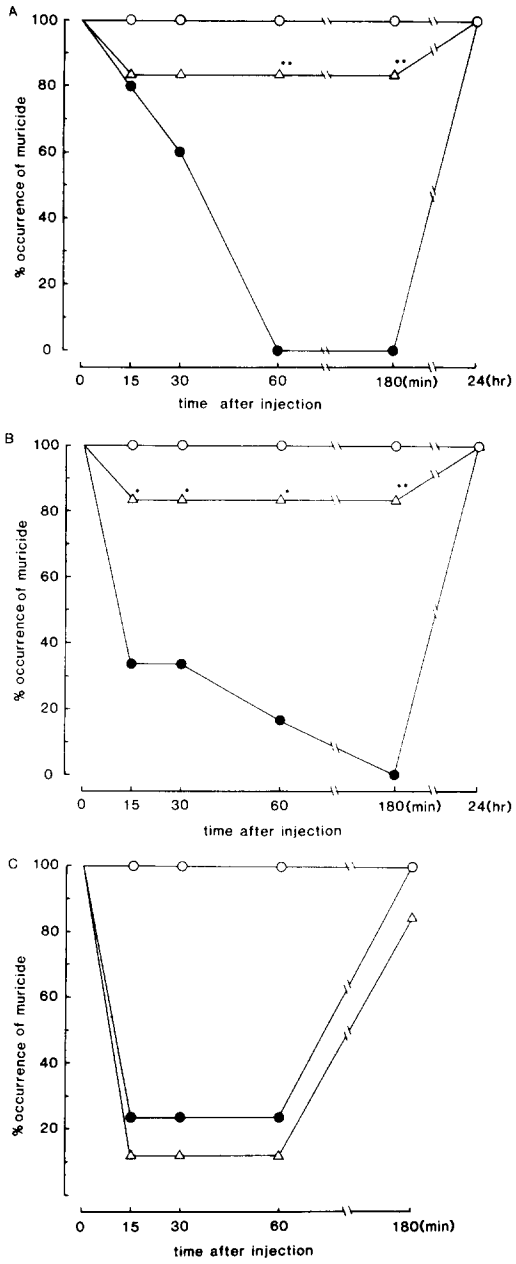

FIG. 4. A: The effect of DMI and 6-OHDA on suppression by dopamine of muricide induced by thiamine deficiency. DMI (25 mg/kg IP) was pretreated 30 min before ICV injection of 6-OHDA, and 6-OHDA 100 μg/rat ICV was pretreatment 7 days before ICV injection of dopamine (10 μg/rat). Number of animals per group: 6. DMI + 6-OHDA + Saline (○); Saline + Saline + DA (●); DMI + 6-OHDA + DA (△). *$p<0.05$ when compared with saline + saline + DA treated group. **$p<0.01$ when compared with saline + saline + DA treated group. B: The effect of DMI and 6-OHDA on suppression by nomifensine of muricide induced by thiamine deficiency. DMI 25 mg/kg IP was pretreated 30 min before ICV injection of 6-OHDA, and 6-OHDA (100 μg/rat ICV) was pretreated 7 days before ICV injection of nomifensine (10 μg/rat). Number of animals per each group: 6. DMI + 6-OHDA + Saline (○). Saline + Saline + NF (●); DMI + 6-OHDA + NF (△). *$p<0.05$ when compared with saline + saline + NF treated group. **$p<0.01$ when compared with saline + saline + NF treated group. C: The effect of p-CPA on suppression by nomifensine of muricide induced by thiamine deficiency. p-CPA (100 mg/kg IP) was pretreated before IP injection of nomifensine 10 mg/kg. Number of animals per each group: 6. p-CPA 2 days + Saline (○); Saline 2 days + NF (●); p-CPA 2 days + NF (△). When p-CPA + NF treated group was compared with saline + NF treated group, it was not a significant difference (see the Results Section).

the suppressive effect of NF was not influenced by pretreatment with p-CPA (Fig. 4C).

DISCUSSION

Colins *et al.* has reported that destruction of the tissue occurs in medulla oblongata superior flower of forth ventricle quartus, nucleus vestibularis lateralis and near by tegmentum in thiamine deficient rats [2, 14, 17]. They have also observed that chronic administration of pyrithiamine, a thiamine antagonist, causes alteration in tissue in hippocampus, corpus mamillare, globus pallidus and amygdala as well as the above mentioned areas [6]. Moreover, dopaminergic ascending fibers project from ventral tegmentum to amygdala [8], and Nakamura *et al.* have reported that microinjection of 6-OHDA into ventral tegmentum caused the muricide response in rats [9]. These reports suggest that alterations of areas within amygdala and hippocampus caused by thiamine deficiency are related to muricide activity. So, we examined the relationship between the thiamine deficiency-induced muricide activity and the dopaminergic system in the CNS by the administration of L-dopa, DA and NF, a DA uptake inhibitor. Chronic administration of L-dopa and NF during feeding of thiamine deficient diet attenuated the muricide response. In addition, acute administration of either drug suppressed the muricide activity in thiamine deficient rats dose-dependently. These results suggest that the catecholaminergic system may have an important role in the regulating of the thiamine deficiency-induced muricide response. Following results contained in this report suggest that the suppression of muricidal activity induced by thiamine deficiency is really due to increased DA activity in the CNS. (1) The suppressive effect of L-dopa was markedly augmented by the addition of Ro4-4602 (5 mg/kg), which inhibited the peripheral dopa decarboxylase. (2) Injection of DA ICV was effective in suppressing the muricide induced by thiamine deficiency. This suppression showed a dose-dependency. These results strongly suggest that the suppressive effect of L-dopa is not due to peripheral, but central dopaminergic mechanism.

Small doses of apomorphine and L-dopa can cause sedation and hypokinesia, which are antagonized by neuroleptics in doses too low to block postsynaptic receptors [5]. Therefore, it has been postulated that, in contrast to the postsynaptic activity of larger doses of these drugs, the effect of small doses of these drugs to decrease dopaminergic function may be specifically mediated via DA receptors. In these experiments, apomorphine in small doses which may stimulate presynaptic DA receptors suppressed the muricide activity induced by thiamine deficiency. Moreover, the suppressive effect of DA administered ICV was antagonized by pretreatment with DMI + 6-OHDA. Thus, the present results indicate that DA and apomorphine may suppress the muricidal activity by decreasing the dopaminergic activity via stimulation of the presynaptic DA receptors as suggested by Castentin *et al.* [3]. NF has been shown in vitro to inhibit the reuptake of NA and has an equivalent activity to nortryptiline. In this respect, it has a similar but less pronounced effect on 5-HT reuptake [15]. The tricyclics do not interfere with DA reuptake, whereas NF is even more active in vitro than the potent inhibitors of DA reuptake such as amphetamine [7]. In this study, NF suppressed the muricide activity in a dose-dependent manner as previously reported for amitryptyrine, chlomipramine and desipramine [12]. If the activity of NF were mediated via inhibition of NE reup-

take, the suppression of muricide activity would not be abolished by pretreatment with DMI + 6-OHDA. If its effect were mediated via inhibition of 5-HT reuptake, the suppressive effect would be abolished by pretreatment with p-CPA. However, in this study, p-CPA did not abolish the suppressive effect of NF for muricide. There is some evidence that NF possesses a direct postsynaptic receptor-activating effect [3]. However, if NF were acting on postsynaptic DA receptors, the suppression of muricide activity would not be abolished by the pretreatment with DMI + 6-OHDA. Therefore, the suppressive effect of NF appears to be attributed to the inhibition of the DA reuptake activity.

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