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The α-Naphthoxyacetic Acid-Elicited Retching Involves Dopaminergic Inhibition in Mice

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FURUKAWA, T. AND K. YAMADA. *The α-naphthoxyacetic acid-elicited retching involves dopaminergic inhibition in mice.* PHARMAC. BIOCHEM. BEHAV. 12(5) 735–738, 1980.—Alpha-naphthoxyacetic acid (α-NOAA), one of the jumping-inducers, elicited a dose-dependent retching behavior at doses ranging from 250 to 550 mg/kg in mice and vomiting at a dose of 550 mg/kg in pigeons. Protoveratrine-A (PV-A, 0.1 mg/kg), a veratrum alkaloid, also induced retching in mice and vomiting in pigeons, while apomorphine (2 mg/kg) produced neither retching in mice nor vomiting in pigeons though it induced feeding in pigeons. The retching elicited by α-NOAA or PV-A was not significantly affected by scopolamine, aminooxyacetic acid and γ-butyrolactone, but was markedly inhibited by apomorphine (2 mg/kg), this inhibitory effect being antagonized without significance by haloperidol which did not itself augment the retching. These results imply that the retching elicited by α-NOAA or PV-A seems to involve at least in part an inhibition of dopaminergic neuron activity.

Retching Vomiting α-Naphthoxyacetic acid Protoveratrine-A Apomorphine Dopaminergic neurons

A PECULIAR retching behavior in mice is characterized by recurrent episodes of wide opening of the mouth [10]. The administration of appropriate doses of veratrum ester alkaloids consistently cause retching in mice [7,10]. There is a report that protoveratrine-A (PV-A), a veratrum ester alkaloid, induces a decrease of 5-hydroxyindoleacetic acid in the brain, a decrease of serotonin and 5-hydroxyindoleacetic acid in the heart and an increase of norepinephrine in the spleen [6]. However, no definite influence on the retching was exerted by the pretreatment with any drugs modifying endogenous monoamine levels, such as reserpine, α-methyl-p-tyrosine, pargyline, l-dopa, p-chlorophenylalanine or 5-hydroxytryptophan [6]. In addition, premedication with various drugs, such as chlorpromazine, atropine, morphine, phenobarbital or bromide did not prevent the retching [10]. As the specific depressant for the PV-A-induced retching has been thus unknown, the neurological mechanism involved in retching still remains to be elucidated.

On the other hand, α-naphthoxyacetic acid (α-NOAA), used in the present experiments, is known to elicit jumping behavior [11,13].

We found that α-NOAA did elicit retching behavior in mice and the neurological mechanism involved in this behavior was investigated.

METHOD

Animals

Healthy ddY male albino mice obtained from Kyudo Animal Laboratory (Kumamoto, Japan) were used, the body weights being 28–33 g at the beginning of the experiment. The food consisted of mouse food, Oriental Yeast Ltd, and the mice were permitted food and water ad lib except during trials. Adult pigeons *(Columba livia domestica)* weighing 400–450 g obtained from Kyudo Animal Laboratory (Kumamoto, Japan) were also used. All trials and breeding were carried out at an environmental temperature of 23 ± 1°C and a humidity of 50 ± 10%.

Behavioral Observation

Groups of 5 mice were placed in plastic boxes (33 cm long×25 cm wide×11 cm high), the bottom of which was laid with paper hand towels. The number of retches was counted for a 30-min period after α-NOAA or a 60-min period after PV-A and apomorphine.

Vomiting responses in pigeons were observed in wire net cages (47 cm long×33 cm wide×18 cm high).

Administration of Drugs

The mice received intraperitoneal injections of α-NOAA at doses ranging from 250 to 550 mg/kg, PV-A at a dose of 0.1 mg/kg, apomorphine at doses of 0.05 and 2 mg/kg or saline as a control. The volumes of drug injected were 0.1 ml/10 g. In the experiments on influences of various drugs on the retching behavior, the respective time interval between pretreatment with following drugs and injection of the retching-inducer such as α-NOAA (550 mg/kg, IP) and PV-A (0.1 mg/kg, IP) was 8 hr for aminooxyacetic acid (30 mg/kg, SC), 45 min for γ-butyrolactone (100 mg/kg, IP), 30 min for scopolamine (5 mg/kg, IP) and 5 min for apomorphine (2 mg/kg, IP). In combined administration of haloperidol with apomorphine, haloperidol (2 mg/kg, IP) was administered 30 min and apomorphine (2 mg/kg, IP) 5 min before injection of the retching-inducer.

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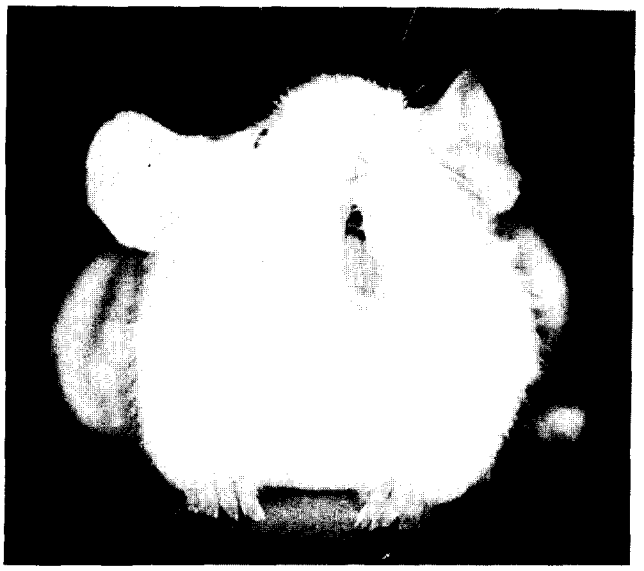

FIG. 1. The retching produced by α-naphthoxyacetic acid (α-NOAA), 550 mg/kg, IP, in a male mouse.

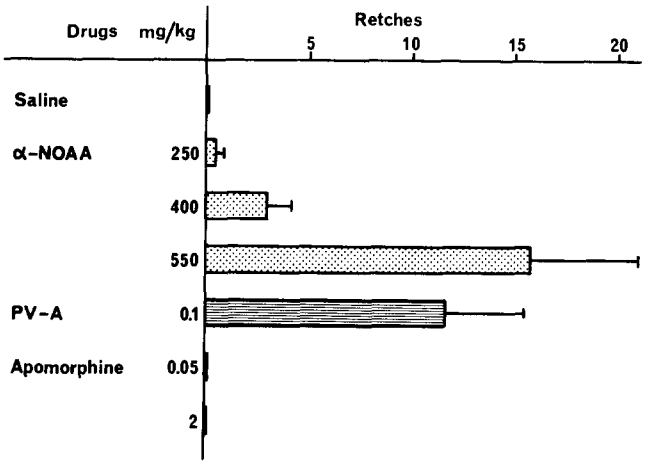

FIG. 2. The retching induced by α-NOAA, protoveratrine-A (PV-A) or apomorphine. Retches were counted for 30 min after α-NOAA or 60 min after PV-A and apomorphine. Each value is the mean (±SEM) of 10 mice. Statistics were done by a one-way analysis of variance followed by the Dunnett's test. The analysis of variance yielded a significant groups effect, $F(6,63)=8.77$, $p<0.01$. The Dunnett's test revealed significant differences at α-NOAA (550 mg/kg) group ($t=5.06$, $p<0.01$) and PV-A group ($t=3.71$, $p<0.01$) from the saline group.

To pigeons, α-NOAA (550 mg/kg, SC), PV-A (0.1 mg/kg, SC) or apomorphine (2 mg/kg, SC) were injected in a volume of 0.5 ml/100 g.

Drugs

The drugs used were α-naphthoxyacetic acid (Tokyo Kasei Chemicals), protoveratrine-A (Sigma), apomorphine hydrochloride (Sandoz A. G.), haloperidol (Serenace, Dainippon Pharmaceutical), scopolamine hydrobromide (Nakarai Chemicals), aminooxyacetic acid hemi-hydrochloride (Sigma), γ-butyrolactone (Nakarai Chemicals) and carboxymethyl cellulose (Ishizu Pharmaceutical). Alpha-NOAA and PV-A were administered as a suspension in 0.25% carboxymethyl cellulose. The other drugs were dissolved in distilled water. As control studies, injections of 0.25% carboxymethyl cellulose, distilled water or saline did not exhibit any abnormal behaviors including retching.

Statistical Analysis

Retching responses were expressed as mean values ± standard errors of the mean, and statistical analysis was calculated using a one-way analysis of variance, the Dunnett's test and the Tukey's test [12]. The level of significance chosen was $p<0.05$.

RESULTS

The Retching Induced by α-NOAA, PV-A or Apomorphine in Mice

As shown in Fig. 1, α-NOAA induced retching which was a peculiar behavior characterized by recurrent episodes of wide opening of the mouth. Each retch was accompanied by salivation and lacrimation, and sometimes by a sudden jumping. Retching began within about 5 min and was most prominent 10 to 15 min after active doses of α-NOAA, ceasing usually within 30 min. After 30 min, the mice were markedly depressed and no more retched at any doses. As seen in Fig. 2, the response to α-NOAA was dose-dependent at doses ranging from 250 to 550 mg/kg. At a dose of 550 mg/kg, the percent incidence of retching among mice tested was 100% and the number of retches during a 30-min period was 15.8 ± 4.2 (n=10). This dose of α-NOAA was regularly used for the following drug antagonism studies.

On the other hand, PV-A elicited retching which was a downward and quick opening of the mouth, with upward and downward movement of the thoracic skin. Each retch was preceded by a decrease in exploratory behavior, salivation and preening. At a dose of 0.1 mg/kg, the percent incidence of retching was 100%, and the number of retches 11.6 ± 3.8 (n=10) during a 60-min period (Fig. 2), retching being most prominent 20 to 30 min after administration of PV-A.

Apomorphine, at doses of 0.05 and 2 mg/kg, did not cause a peculiar syndrome characterized by opening of the mouth like retching (Fig. 2). However, typical symptoms of behavioral excitation (hypermotility, rearing, stereotypy) were seen after the injection of apomorphine (2 mg/kg), and penile erection was occasionally observed at a low dose (0.05 mg/kg).

Influences of Certain Drugs on the Retching Induced by α-NOAA or PV-A

As shown in Fig. 3, apomorphine, at a dose of 2 mg/kg, markedly inhibited the α-NOAA-induced retching responses. This inhibitory effect of apomorphine on the retching was antagonized but not significantly by pretreatment with haloperidol (2 mg/kg). However, treatment with haloperidol (2 mg/kg), scopolamine (5 mg/kg), aminooxyacetic acid (30 mg/kg) or γ-butyrolactone (100 mg/kg) failed to affect the retching.

As seen in Fig. 4, the retching responses induced by PV-A were also inhibited by apomorphine (2 mg/kg), and this inhibition was antagonized but not significantly by pretreatment with haloperidol (2 mg/kg). Whereas, haloperidol (2 mg/kg),

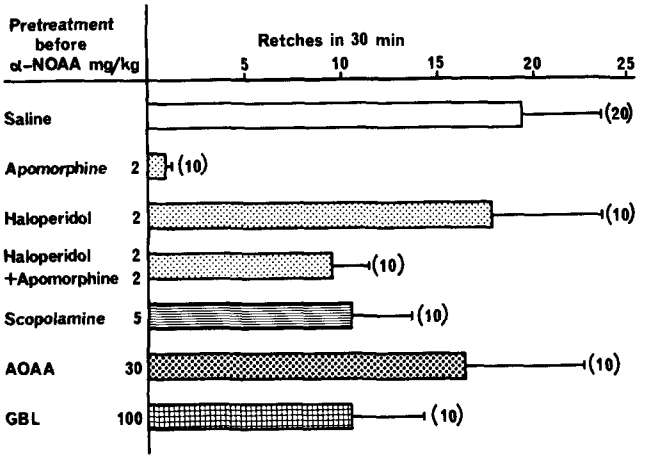

FIG. 3. Influences of apomorphine, haloperidol, haloperidol plus apomorphine, scopolamine, aminooxyacetic acid (AOAA) and γ-butyrolactone (GBL) on the α-NOAA (550 mg/kg)-elicited retching. Retches were counted for 30 min after α-NOAA. Each value is the mean (±SEM). Numbers in parentheses indicate numbers of mice used. Statistics were done by a one-way analysis of variance followed by the Tukey's test. The analysis of variance yielded a significant groups effect for the four groups (saline, apomorphine, haloperidol, and haloperidol plus apomorphine), $F(3,46)=3.70$, $p<0.05$. The Tukey's test revealed that the difference between the apomorphine group and the saline group was significant ($p<0.05$), whereas the other groups differences were not.

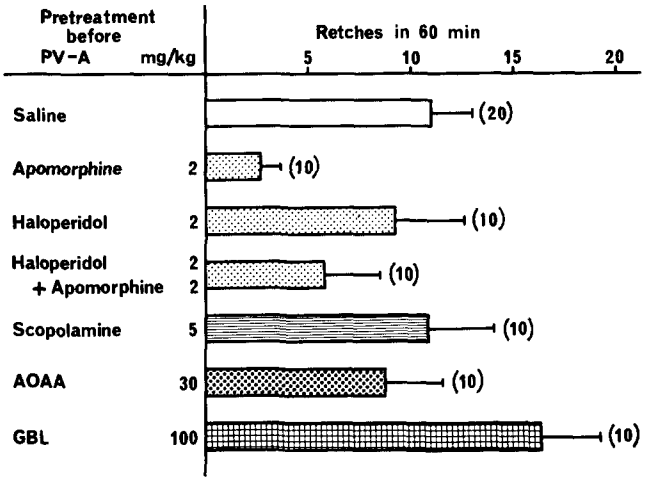

FIG. 4. Influences of apomorphine, haloperidol, haloperidol plus apomorphine, scopolamine, aminooxyacetic acid (AOAA), and γ-butyrolactone (GBL) on the PV-A (0.1 mg/kg)-elicited retching. Retches were counted for 60 min after PV-A. Each value is the mean (±SEM). Numbers in parentheses indicate numbers of mice used. Statistics were done by a one-way analysis of variance followed by the Tukey's test. The analysis of variance yielded a significant groups effect for the four groups (saline, apomorphine, haloperidol, and haloperidol plus apomorphine), $F(3,46)=2.83$, $p<0.05$. The Tukey's test revealed that the difference between the apomorphine group and the saline group was significant ($p<0.05$), whereas the other groups differences were not.

scopolamine (5 mg/kg), aminooxyacetic acid (30 mg/kg) and γ-butyrolactone (100 mg/kg) did not inhibit the retching responses induced by PV-A.

Vomiting after α-NOAA, PV-A or Apomorphine in Pigeons

The subcutaneous injection of α-NOAA (550 mg/kg, n=2) or PV-A (0.1 mg/kg, n=2) to pigeons caused frequent and violent vomitings in all cases. The vomitings began 5 to 10 min and lasted 30 min after injection of these drugs. However apomorphine (2 mg/kg, n=2) did not elicit vomiting although it induced marked feeding (pecking) in pigeons.

DISCUSSION

A characteristic retching behavior occurred after administration of α-NOAA in mice. This α-NOAA-induced retching behavior was not inhibited by scopolamine, an anticholinergic drug, aminooxyacetic acid, an inhibitor of GABA-transaminase, or γ-butyrolactone, a precursor of γ-hydroxybutyrate, suggesting that the retching does not involve either cholinergic or GABAergic mechanism. However, apomorphine (2 mg/kg) markedly inhibited the retching, and this inhibitory effect was antagonized by haloperidol, a dopamine specific antagonist, though not significantly. The α-NOAA (700 mg/kg)-elicited jumping behavior in mice was also inhibited by apomorphine and this inhibition was significantly antagonized by previous administration of haloperidol [13]. Our recent result showed that intraperitoneal injections of α-NOAA at doses of 550 and 700 mg/kg in mice exhibited a significant increase of brain dopamine levels (unpublished data). As to the PV-A-induced retching, haloperidol, scopolamine, aminooxyacetic acid and γ-butyrolactone did not affect, but apomorphine also blocked the retching, this inhibitory effect being antagonized by haloperidol though not significantly. It has been reported that low doses (from 0.025 to 0.2 mg/kg) of apomorphine preferentially activate presynaptic dopamine autoreceptors which results in an inhibition of dopamine release and a consequent decrease of its synthesis, whereas higher doses (from 0.4 to 3.2 mg/kg) of apomorphine stimulate postsynaptic dopamine receptors [2, 3, 9, 14]. At a dose (2 mg/kg) used in the present experiments, apomorphine seems to act mainly on the postsynaptic dopamine receptor sites. These results, therefore, may imply that the activation of postsynaptic dopamine receptors by apomorphine may be involved in its inhibitory effect on the α-NOAA-induced retching behavior. However, haloperidol antagonized to a certain degree the inhibition by apomorphine in retching behavior, while it did not itself potentiate the retching responses. For some of the imperfect results, there is no obvious explanation. But, haloperidol acts in complicated manner on the dopaminergic neuron activities. It blocks pre- and postsynaptic dopamine receptors as well as inhibitory and excitatory dopamine receptors [4, 8, 14]. It might be probable that, provided that dopamine receptors are inordinately stimulated by administration of apomorphine, blocking action of haloperidol on these receptors is manifested, so far as the retching responses are concerned. Accordingly, the retching seems to involve at least in part an inhibition of dopaminergic neuron activity, perhaps as a consequence of decrease of dopamine release from the presynaptic sites, though true picture of retching behavior is probably somewhat more complex.

It is well known that apomorphine elicits vomiting by acting on the chemoreceptor trigger zone in cats and dogs [1,5]. In this study, apomorphine, at a dose of 2 mg/kg, did

not elicit vomiting in pigeons, and it did not produce retching in mice, either, as reported previously by Koga and Takemoto [6]. On the contrary, PV-A, which elicited retching in mice, caused vomiting in pigeons as reported before [1,7]. This veratrum alkaloid-induced vomiting was proposed to be due to an excitation of vomiting center resulted from a reflex action originating in a receptor area which was present in close proximity to the nodose ganglia of the vagus nerves [1,7]. Alpha-NOAA was herein demonstrated to be capable of inducing vomiting in pigeons. Therefore, differences in the effects of apomorphine, PV-A and α-NOAA on retching and vomiting might be attributable to their differential effects on the chemoreceptor trigger zone or the nodose ganglia of the vagus nerves.

The present results suggest that α-NOAA can elicit retching in mice and vomiting in pigeons, and the inhibition of the dopaminergic neuron activity seems to be involved in the retching behavior.

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