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Scopolamine-induced convulsions in food given to fasted mice: effects of clonidine and tizanidine

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Abstract

We recently reported that scopolamine pretreated mice fasted for 48 h developed clonic convulsions soon after they were allowed to eat ad libidum. Pretreatment with MK-801, the non-competitive NMDA antagonist, decreased the incidence of these convulsions. We suggested that a possible scopolamine-induced glutamatergic hyperactivity could account for these convulsions. Using α_2-agonists, clonidine, which has been shown to inhibit glutamate release, and tizanidine, the present study was performed to find some additional data for the role of glutamate in the underlying mechanism of scopolamine-induced convulsions in food given fasted mice. Animals fasted for 48 h and pretreated (i.p.) with saline, clonidine (0.05, 0.10, 1 mg/kg) or tizanidine (0.10, 0.15, 0.30, 0.45 mg/kg) were treated (i.p.) with either saline or scopolamine (3 mg/kg). Then 20 min later, they were allowed to eat ad libidum and were observed for 30 min for the incidence and onset of clonic convulsions. All doses of clonidine pretreatment completely suppressed (0%) scopolamine-induced clonic convulsions (75%). On the other hand, only 0.15 mg/kg tizanidine pretreatment significantly decreased (15%) the incidence of convulsions; however as well as 0.15 mg/kg, both 0.30 and 0.45 mg/kg tizanidine pretreatments significantly increased latency to the onset of convulsions.

Keywords: Scopolamine; Clonidine; Tizanidine; Fasting; Convulsion; Food; Mice

1. Introduction

During the training sessions of the experiments investigating the effects of scopolamine on memory and learning processes in mice, we observed that some of the fasted animals exhibited seizures soon after finding and starting to eat the food pellet in the maze. We then reported that mice treated with 1 or 3 mg/kg scopolamine after fasting for 48 h developed clonic convulsions when were allowed to eat a small amount of food for

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30 s or ad libidum (Enginar et al., 1996, 1997; Yamantürk et al., 1996). The additive effect of scopolamine pretreatment and access to food was essential in the induction of convulsions. However, it was not clear whether scopolamine precipitated seizures in food given fasted mice or eating precipitated seizures in scopolamine pretreated fasted mice.

Interestingly, scopolamine has indeed anticonvulsive activity. This anticholinergic drug reverses convulsions elicited by anticholinesterase, soman (Anderson et al., 1994) and muscarinic agonists, muscarine (Beleslin and Samardzic, 1979) and pilocarpine (Turski et al., 1984; Smolders et al., 1997). It is suggested that scopolamine, as a muscarinic antagonist, blocks postsynaptic muscarinic receptors and produces its anticonvulsive activity via reducing cholinergic transmission. However, it is well documented that scopolamine, acting at the presynaptic muscarinic receptors on the cholinergic receptors, also enhances acetylcholine release (Molenear and Polak, 1980; Bymaster et al., 1993; Dixon et al., 1997). Thereby, enhancement of acetylcholine release would be associated with increased transmission at the cholinergic synapses and cholinergic hyperactivity may lead to convulsions in scopolamine pretreated fasted animals. On the other hand, scopolamine-induced glutamatergic hyperactivity is also possible, since scopolamine may enhance glutamatergic transmission via antagonizing the presynaptic cholinergic inhibition on glutamate release (Williams and Johnston, 1990; Sim and Griffith, 1996). Hyperactivity in the glutamatergic system may indirectly contribute to convulsive activity, since glutamate regulates acetylcholine output in different brain regions through *N*-methyl-D-aspartate (NMDA) receptors (Lodge and Johnston, 1985; Ulus et al., 1992) and MK-801 (dizocilpine), the non-competitive NMDA antagonist, prevents convulsions (Savolainen and Hirvonen, 1992) and arrests status epilepticus (Sparenborg et al., 1992) produced by cholinergic drugs, or may directly contribute to convulsive activity, since activation of NMDA receptors produces convulsions (Czuczwar et al., 1985; Dunn and Corbett, 1992). There are reports suggesting that cholinergic convulsions are due to glutamate release and subsequent glutamate receptor activation (Wade et al., 1987) and cholinergic system is responsible for seizure onset and maintenance and driving glutamatergic mechanisms to support sustained seizure activity (Turski et al., 1989; Smolders et al., 1997). Thus, cholinergic and/or glutamatergic hyperactivity may play a role(s) in the underlying mechanisms of these convulsions in the scopolamine pretreated food given fasted animals.

We suggested that finding food and/or eating after prolonged fasting makes animal more vulnerable to a convulsive activity and speculated that during fasting for 48 h an adaptation in the central nervous system might occur to compensate the effect(s) of food deprivation. Such an adaptation might induce a decrease in the release of acetylcholine or glutamate leading to an up-regulation in the receptors. Finding food and eating may produce a change in neurochemical or endocrine systems, a sympathetic activation, for example, that leads to an increase in the release of acetylcholine or glutamate that might facilitate the putative convulsive effect of scopolamine. Our findings showed that blockade of NMDA receptors by MK-801 decreased (Enginar et al., 1997) and pretreatment with physostigmine, the cholinesterase inhibitor, potentiated the incidence of convulsions, respectively. The effect of physostigmine, however, was significant only when fasted mice fed restrictedly (Yamantürk et al., 1996), not ad libidum (Enginar et al., 1997). On the other hand, pretreatment with atropin, another anticholinergic drug, also induces convulsions in food given fasted mice (unpublished results).

It was reported that activation of α_2-adrenoceptors located on the nerve terminals of glutamatergic neurons inhibit the release of glutamate (Kamisaki et al., 1991, 1992; Bickler and Hansen, 1996). A decrease in the glutamatergic transmission via inhibition of glutamate release may also prevent the occurrence of convulsions, as did blockade of its receptors by MK-801. Thus, using clonidine and tizanidine, as α_2-agonists, the present study was performed to find some additional data for the role of glutamatergic hyperactivity in the underlying mechanism of scopolamine-induced convulsions in food given fasted mice.

2. Materials and methods

Inbred male albino mice weighing 24–30 g were used. They were housed under standard laboratory conditions for at least 1 week prior to experimentation and allowed free access to both food and water. Experiments were carried out between 09:30 and 14:30 h in a temperature controlled (22 ± 2°C) quiet room. Each mouse was used once in the experiments.

2.1. Procedure

Mice were weighed and food deprivation was started 2 days before the experiments. Water was freely available. On the day of testing, animals fasted for 48 h were weighed, divided into eight groups and treated with saline (control), clonidine (0.05, 0.10, 1 mg/kg) or tizanidine (0.10, 0.15, 0.30, 0.45 mg/kg). Then, 10 min later, animals in each group were given either saline or 3 mg/kg scopolamine. Following treatments, all animals were individually placed in wire mesh cages. After 20 min, they were given five big pieces of food pellets, about 2 g, and allowed to eat ad libitum until the end of the experiments. All animals were observed for 30 min for the incidence and onset of clonic convulsions. The first signs of a seizure activity were freezing and gustatory movements that were followed by forelimb cloni. Some of the fasted animals exhibited generalized convulsions with rearing, falling down and jumping. A convulsive response was assessed as forelimb cloni with rearing. Observers were blind to the treatments.

2.2. Drugs

Scopolamine hydrobromide was purchased from Sigma, St. Louis, MO. Clonidine hydrochloride and tizanidine hydrochloride were generous gifts from Boehringer Ingelheim and Sandoz, respectively. Drugs were dissolved in saline and injected intraperitoneally (i.p.) in a volume of 0.1 ml per 25 g body weight.

2.3. Statistical analysis

Chi-square ($n > 20$) and Fisher's Exact test ($n < 20$) were used to evaluate the frequency of the incidence of convulsions. The onset of convulsions was evaluated with Student's t-test.

3. Results

The body weights of the mice fell to approximately 80–85% of the starting body weights after fasting for 48 h.

The effects of clonidine and tizanidine on scopolamine-induced convulsions in fasted mice are shown in Table 1. Scopolamine treatment alone caused convulsions in fasted animals and this effect was statistically significant ($P < 0.01$) when compared with the saline injected control group. Pretreatment with clonidine, 0.05, 0.10 and 1 mg/kg, completely suppressed ($P < 0.01$) and pretreatment with tizanidine, only at a dose of 0.15 mg/kg, decreased ($P < 0.05$) the incidence of scopolamine-induced convulsions. Latency to the onset of convulsions, on the other hand, was increased by pretreatments with 0.15 ($P < 0.01$), 0.30 ($P < 0.01$) and 0.45 ($P < 0.05$) mg/kg tizanidine.

None of the animals in the groups treated with clonidine or tizanidine with saline developed convulsions (data not shown).

4. Discussion

In the literature there are reports of epileptic seizures evoked by eating (Koul, 1991; Mandal et al., 1992; Senanayake, 1994). The type of epilepsy was partial and complex partial seizure, secondarily generalized in the majority of the patients, was the commonest form encountered. Reported cases suggest that various mechanisms such as chewing, swallowing or the complete act of eating a meal or conditioned reflex may act as triggering factors in this form of reflex epilepsy. In the fasted animals, the first signs of a seizure activity were freezing and automatic gustatory movements that were followed by forelimb cloni which might develop to secondarily generalized convulsions with rearing, falling down and jumping. In respect to

similarities in triggering factors and manifestations of the seizure activity, our experiments may provide insight into the mechanism of eating epilepsy.

This study demonstrated that scopolamine-induced convulsions in food given fasted mice were completely suppressed by clonidine and either decreased or delayed by tizanidine. Although a direct or indirect effect via another system(s) cannot be excluded, suppression of convulsions by these two α_2-agonist drugs may be suggested to be dependent on their inhibitory effect on glutamate release, since clonidine has been shown to inhibit the K^+-evoked glutamate overflow through presynaptic α_2-adrenoceptors in various brain regions (Kamisaki et al., 1991, 1992; Bickler and Hansen, 1996). This suggestion is supported by the studies which have showed that reduction of the epileptic activity in the mouse entorhinal cortex (Pralong and Magistretti, 1995) and in the rat basolateral amygdala (Ferry et al., 1997) are due to glutamatergic hypoactivity produced by activation of α_2-adrenergic receptors by noradrenaline. On the other hand, clonidine (Ernsberger et al., 1987; Muramatsu and Kigoshi, 1992) and tizanidine (Muramatsu and Kigoshi, 1992) also bind to imidazoline receptors as well as α_2-adrenoceptors. However, the contribution of imidazoline receptors to the inhibition of glutamate release seems invalid, since data confirm that the heteroreceptors which inhibit the K^+-evoked glutamate overflow are α_2-adrenoceptors, not imidazoline receptors, in the brain regions where the presynaptic α_2-adrenoceptors which regulate the release of glutamate are mainly distributed (Kamisaki et al., 1992). On the other hand, tizanidine was not found as effective as clonidine in preventing convulsions. In addition, a clear-cut dose-related enhancement of inhibition of convulsions with tizanidine was not observed, since in higher doses tizanidine was only effective in delaying the onset of convulsions. A possible explanation for this discrepancy between clonidine and tizanidine may depend upon their affinities to α_2-adrenergic and imidazoline receptors. Tizanidine binds to the imidazoline receptors with approximately 20 times higher affinity than the α_2-adrenoceptors, whereas the affinity of clonidine for both receptors is equal (Muramatsu and Kigoshi, 1992) or even greater for the α_2-adrenergic receptors than that for the imidazoline receptors (Michel et al., 1989; Piletz et al., 1991). On

Table 1
Effects of various doses of clonidine and tizanidine pretreatment on the incidence and onset of scopolamine-induced clonic convulsions in food given fasted mice[a]

Groups (n)	Clonic convulsions		Number of deaths[b]
	Incidence (%)	Time of onset (min) (mean ± S.E.)	
Control (8)	0	–	–
Scopolamine (13)	75‡	7.85 ± 2.21	3
Clonidine (0.05) + scopolamine (6)	0**	–	–
Clonidine (0.10) + scopolamine (6)	0**	–	–
Clonidine (1) + scopolamine (6)	0**	–	–
Tizanidine (0.10) + scopolamine (11)	45	14.60 ± 4.21	1
Tizanidine (0.15) + scopolamine (10)	10*	16.00 ± 0.00††	–
Tizanidine (0.30) + scopolamine (11)	73	19.68 ± 2.74††	2
Tizanidine (0.45) + scopolamine (10)	40	19.12 ± 3.89†	1

[a] Mice fasted for 48 h were injected i.p. with saline, clonidine (0.05, 0.10, 1 mg/kg) or tizanidine (0.10, 0.15, 0.30, 0.45 mg/kg) 10 min prior to i.p. saline or scopolamine (3 mg/kg) treatments and given free access to food 20 min after the second injections.

[b] Caused by generalized tonic-clonic convulsions developing from clonic convulsions.

‡ $P<0.01$, significantly different from control (saline) group, χ^2-test.

* $P<0.05$, ** $P<0.01$, significantly different from scopolamine group, Fisher's Exact test or χ^2-test.

† $P<0.05$, †† $P<0.01$, significantly different from scopolamine group, Student's t-test

the other hand, it may also be possible that due to a limited half-life, the effect of tizanidine was small in comparison to clonidine.

In our previous study, we showed that pentylenetetrazol convulsions in mice were not prevented by 0.01 and 0.10 mg/kg clonidine, and were even potentiated at a dose of 1 mg/kg (Enginar and Koyuncuoğlu, 1995). Contradictory findings, on the other hand, showed that i.p. administration of up to 0.5 mg/kg or microinfusion in the vicinity of locus ceruleus of clonidine exerted anticonvulsant effects against seizures induced by pentylenetetrazol in mice and amygdala kindling in rats (Loscher and Czuczwar, 1987), and in the kindling model of epilepsy in kittens (Shouse et al., 1996), respectively. Clonidine is indeed a sedative drug. Single, small doses of clonidine administered to laboratory animals lead to sedation which has been explained by stimulation of inhibitory presynaptic α_2-adrenoceptors (Schmitt, 1977). The sedative effect of clonidine, therefore, may be suggested to contribute to its suppressive effect on scopolamine-induced convulsions in food given fasted mice. On the other hand, the anticonvulsive effect of tizanidine may also be suggested to be due to its myorelaxant effect, which is also mediated by an activation of α_2-adrenergic receptors (Corboz et al., 1991). But, motor performance appeared not disturbed in tizanidine treated animals, since none of them exhibited ataxic.

In conclusion, although additional studies are needed to more precisely describe, depending upon the possibility of an action of clonidine and tizanidine on the glutamatergic system, these results provide additional data for the possible involvement of a glutamatergic hyperactivity in the development of convulsions. In fact, our ongoing studies which investigate the effects of various doses of physostigmine and the differences in the levels of blood glucose and NMDA receptor density will describe the role(s) of hypoglycemia, one of the metabolic derangements that cause convulsions (Fisher, 1989), and cholinergic system as well as glutamatergic system in the underlying mechanism of these convulsions.

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