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SCOPOLAMINE-INDUCED CONVULSIONS IN FASTED RATS AFTER FOOD INTAKE: THE EFFECT OF DURATION OF FOOD DEPRIVATION

AÇ SIÇANLARA SKOPOLAMİN UYGULANMASI VE YEM VERİLMESİ İLE OLUŞAN KONVÜLSİYONLAR: YEM YOKSUNLUĞU SÜRESİNİN ETKİSİ

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ABSTRACT

Objective: Mice and rats fasted for 2 days and then treated with antimuscarinic drugs, scopolamine or atropine, develop convulsions soon after food intake. It has been demonstrated that deprivation of food for less than 48 h also causes convulsions in mice. Since there are differences in characteristics of the seizures between mice and rats, the present study evaluated whether rats also develop convulsions after being deprived of food for 3-24 h.

Material and Method: Rats were deprived of food for 3, 6, 18, 24 and 52 h. At the time of testing, the animals were treated i.p. with saline or 3 mg/kg scopolamine and given food 20 min later. All animals were observed for 30 min, checking for the incidence and onset of convulsions.

Results: The fasted animals treated with scopolamine developed convulsions after food intake. The incidence of convulsions was statistically significant in the 6, 18, 24 and 52 h deprived animals. However, neither the incidence nor the latency to seizures showed differences with respect to duration of food deprivation or fasting-induced weight loss.

Conclusion: The present results demonstrate that the scopolamine treated rats that fasted for 24 h or less developed seizures after refeeding intake, indicating that food deprivation in itself, rather than its duration, seems to be the principal factor in the development of these convulsions.

Keywords: Scopolamine, convulsion, food deprivation, rat

ÖZET

Amaç: İki gün aç bırakıldıktan sonra antimuskarinik, skopolamin veya atropin, uygulanan fare ve sıçanlar yeniden yedikten hemen sonra konvülsiyon geçirmektedirler. Farelerin 48 saatten daha kısa süre yemden yoksun bırakılmasıyla da konvülsiyon oluştuğu gösterilmiştir. Fare ve sıçanlar arasında nöbet özelliklerinde farklar olduğu için, bu çalışmada 3-24 saat açlık sonrası sıçanlarda konvülsiyon oluşumu araştırılmıştır.

Gereç ve Yöntem: Sıçanlar 3, 6, 18, 24 ve 52 saat yemden yoksun bırakıldı. Açlık sonrası, hayvanlara serum fizyolojik veya 3 mg/kg skopolamin i.p. uygulandı ve 20 dakika sonra yem verildi. Tüm hayvanlar konvülsiyon sıklığı ve konvülsiyon başlama süresi için 30 dakika izlendi.

Bulgular: Skopolamin uygulanan aç hayvanlar yem yedikten sonra konvülsiyon geçirdi. Konvülsiyon sıklığı 6, 18, 24 ve 52 saat yemden yoksun bırakılan hayvanlarda istatistiksel olarak anlamlı bulundu. Ancak nöbet sıklığı ve başlama süresi açısından, yem yoksunluğu süresi veya açlığa bağlı ağırlık kaybı ile ilişkili bir farklılık ortaya çıkmadı.

Sonuç: Bu sonuçlar, 24 saat veya daha kısa süre aç bırakıldıktan sonra skopolamin uygulanan sıçanlarda yeniden yedikten sonra konvülsiyon oluştuğunu göstererek; sürenin değil, yemden yoksun kalmanın konvülsiyon oluşumunda ana etken olabileceğini işaret etmektedir.

Anahtar Kelimeler: Skopolamin, konvülsiyon, yem yoksunluğu, sıçan

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INTRODUCTION

Mice and rats treated with scopolamine or atropine develop convulsions soon after being allowed to eat ad lib after fasting for 2 days (1, 2). Deprivation of food for 48 h produces significant changes in the kinetics of [^{3}H] glutamate binding in the brain which are partly antagonized by scopolamine treatment and food intake. The additive effect of scopolamine treatment and access to food is essential in the induction of convulsions. All of the complicated acts that occur during eating (e.g. chewing and swallowing movements, smelling and tasting) and stimulation of the amygdala by repetitive oral and masticator movements are suggested to be triggers and underlying factors, because convulsions develop only after solid food intake, but not slurry or fluid feeding (3). Food deprivation in itself, but not its hypoglycemic consequence, seems to be critical in the development of convulsions, because prevention of hypoglycemia by glucose intake during fasting has no preventive effect. Chlorpromazine, haloperidol, noncompetitive N-methyl-D-aspartate (MK-801), clonidine and tizanidine provide effective treatments. However, most of the major and new antiepileptic drugs in clinical use are ineffective in suppressing the seizures (1, 4). Interestingly, the trigger factors and manifestations of the seizures evoked by eating in patients and fasted animals bear some similarities (5).

Various neurochemical changes have been reported in short- and long-term food deprived animals (6-8). Deprivation or restriction of food and subsequent body weight loss has been found sufficient to induce behavioral and cellular effects (6, 9). These findings might be evaluated as deprivation- and/or weight loss-induced contributing factors in the occurrence of convulsions in fasted animals. After fasting for 48 h, mice fall to approximately 80-85% of the starting body weights. Rats are more resistant to fasting and only lose 8-9% after the same period of deprivation. Thus, fasting was prolonged in rats to 52 h to yield at least 10-12% weight loss (2). Mice and rats also differ in seizure stages, response to antiepileptic drugs (2), and strain-related sensitivity to seizures (10). Shortening of the food deprivation period showed that fasting for even brief periods results in seizures in mice (11). The present study was performed to evaluate whether rats also develop convulsions after fasting for varying lengths of time less than 52 h.

MATERIALS AND METHODS

Animals

All studies were approved by the Istanbul University Local Ethics Committee on Animal Experiments (39/05.07.2007) and were in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Experiments

Inbred male Wistar albino rats (280-300 g) were weighed and deprived of food for 3, 6, 18, 24 and 52 h. During the fasting period, animals had free access to water. At the time of testing, animals were reweighed and treated i.p. with 3 mg/kg scopolamine hydrobromide (Sigma, St Louis, MO) or saline (n=7-9) and were then individually placed in wire mesh cages. Twenty minutes later, they were given food pellets and allowed to eat ad libitum. All animals were observed for 30 min for the incidence and onset of convulsions. Stages of seizure activity were; (0) no difference; (1) freezing; (2) tongue protrusion and neck spasms; (3) forelimb clonus; (4) forelimb clonus and tonic activity in the upper part of the body with rearing and/or falling over; (5) generalized convulsions with rearing, falling over and jumping. The animals were also observed for myoclonus of hindlimbs. A convulsive response was assessed as repeated (at least twice) buccal movements with an opening of the mouth and protrusion of the tongue with neck spasms (stage 2+). The onset of convulsions was defined as the time between refeeding and stage 2 activity. The incidence of convulsions was expressed as the percentage of animals displaying either stage 2+, 3, 4 or 5 activity. Experiments were carried out between 08:00 and 15:00 in a temperature controlled (21±2°C) quiet room. Observers were blind to the treatments.

The saline treated control animals did not even exhibit stage 1 activity. Therefore, in the tables, "Control" group refers to control groups in all deprivation regimens. Scopolamine treatment given to the control animals showed no effect on seizure susceptibility, regardless of the food deprivation duration (data not shown).

Statistical analysis

The body weight loss and onset of convulsions data were evaluated using one-way analysis of variance (ANOVA) followed by Bonferroni multiple test and Tukey test, respectively. Fisher's exact test (n<20) was used for the evaluation of the frequency of the incidence of convulsions and seizure stages.

RESULTS

After fasting for 3, 6, 18, 24 and 52 h, the body weights of the animals fell to 98.5, 98.3, 95.5, 93.6 and 89.2% of the initial body weights, respectively. Food deprivation for 18, 24 and 52 h caused significant weight loss $F(4,36)=142.913$; $P<0.001$).

Scopolamine treatment caused convulsions in rats deprived of food for 6, 18, 24 and 52 h. When compared with the saline-treated control group, this effect was statistically significant in the 6-h ($P<0.01$), 18-h ($P<0.01$), 24-h ($P<0.05$) and 52-h ($P<0.05$) deprived groups (Table 1). The incidence of convulsions in the different time periods of deprivation (6, 18, 24 and 52 h) was insignificant when

Table 1: Effect of duration of food deprivation on the incidence and onset of scopolamine-induced convulsions in fasted rats after refeedinga

Groups [n]	Convulsions Incidence (%)	Time of onset (min) (mean±SEM)[b]
Control [9]	0	-
Scopolamine 3 h fasted [7]	0	-
Scopolamine 6 h fasted [7]	86**	6.8±1.7
Scopolamine 18 h fasted [9]	78**	10.1±2.9
Scopolamine 24 h fasted [9]	56*	8.8±3.7
Scopolamine 52 h fasted [9]	56*	7.0±1.5

[a]Rats deprived of food for different periods of time were injected with saline or 3 mg/kg scopolamine and were given free access to food 20 min later.
[b]Calculated from seizing animals.
[n] number of animals
* P<0.05, ** P<0.01, significantly different from control (saline) group, Fisher's Exact test.

Table 2: Number of animals showing seizure stages in each group in the experiment

Groups [n]	Stage 0	Stage 1	Stage 2	Stage 2+	Stage 3	Stage 4	Stage 5	Myoclonus of hindlimbs
Control [9]	9	-	-	-	-	-	-	-
Scopolamine 3 h fasted [7]	-	7	-	-	-	-	-	3
Scopolamine 6 h fasted [7]	-	-	1	-	5	-	1	-
Scopolamine 18 h fasted [9]	1	1	-	4	3	-	-	-
Scopolamine 24 h fasted [9]	1	3	-	4	-	1	-	1
Scopolamine 52 h fasted [9]	-	2	2	1	2	1	1	-

[n] number of animals

compared with each other. ANOVA yielded no statistically significant group effects for the onset of convulsions $F(3,19)=0.384$; $P<0.05$.

The seizure stages that developed in each group are given in Table 2. Myoclonus of hindlimbs occurred in 3 of 7 and 1 of 9 animals in the 3 h and 9 h deprived groups, respectively.

DISCUSSION

The present results demonstrate that rats deprived of food for 6 to 24 h develop convulsions after scopolamine treatment and food intake, as did the 52 h fasted animals. The development of a status-like activity, seizure behavior lasting for minutes, and recurrence of stages were common features in some of the seizing animals in all groups. As reported previously in the 52 h fasted rats (2), repetitive myoclonus of hindlimbs occurred starting before or after food intake in animals deprived of food for 3 or 24 h (Table 2). The percentage of animals displaying repeated buccal movements with an opening of the mouth and protrusion of the tongue with neck spasms (either stage 2+, 3, 4 or 5) was statistically significant in the 6, 18, 24 and 52 h deprived groups. Animals in the 3 h deprived group only displayed stage 1 activity (Table 2). Continuous electroencephalogram (EEG) recordings from rats demonstrated an increased frequency of background activity and high-amplitude irregular spikes and sharp waves during stage 1 (unpublished data). The number of convulsing animals and the latency to develop convulsions was not significantly correlated with the length of food deprivation. Our previous study conducted on mice deprived of food for periods of 1, 2, 3, 6, 9, 12, 18 and 24 h showed that the incidence of convulsions was statistically significant in 2, 3, 12, 18 and 24 h fasted animals. Rats seem less prone to develop convulsions with shorter periods of fasting in comparison with mice because 6 h of fasting was required to reach the convulsive endpoint (stage 2+, 3, 4 or 5 activity).

Fasting for 3-52 h caused 1.5-10.8% body weight loss in rats. A marked effect of food deprivation on weight loss was observed at 18 h and thereafter. A deprivation or restriction of food and subsequent body weight loss

has been found sufficient to induce behavioral and cellular effects (6, 9, 12). However, in the present study, the convulsive response seemed to be unrelated to weight loss induced by fasting, because 6 h deprived animals develop convulsions without significant reductions in body weight. These results confirm previous findings that food deprivation or fasting stress, rather than the magnitude of weight loss, influences susceptibility to seizures (2, 11).

Based upon biochemical and behavioral data, we previously suggested adaptive adjustments in glutamatergic, dopaminergic and cholinergic systems as ways to meet the demands of food deprivation (11). Accordingly, our ongoing studies in mice evaluate fasting-induced M_1 and M_2 muscarinic receptor expression in different brain regions. Various neuroadaptive changes in the brain have been reported in rats deprived of food for 24 or 16-18 h (13, 14). However, the findings in the present study imply neuroadaptations as underlying mechanisms of these convulsions, which may occur even after a few hours of food deprivation in animals. Acute stress elicits a prominent form of synaptic plasticity in midbrain dopamine neurons (15) where basal extracellular dopamine levels are decreased by food deprivation (6). It has been suggested that acetylcholine and glutamate possess regulatory roles in dopaminergic function (16). Although speculative, food deprivation, which is regarded as a stressor, might also produce neuroadaptive changes in mesolimbic dopamine release, which mediates convulsions in fasted animals.

In conclusion, the present study demonstrates that deprivation of food for 6, 12, 18 and 24 h causes convulsions in scopolamine-treated and refed rats. In accordance with those observed in mice, the results indicate that convulsions are primarily affected by food deprivation stress, but not by weight loss, which provides additional data for the underlying mechanism(s) of this convulsion method.

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