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FURTHER EVIDENCE AGAINST USING SORBITANS IN THE BRAIN MECHANISMS INVESTIGATION

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Abstract. In both experimental groups an intraperitoneal injection of a single dose of 450 mg/kg p-Chlorophenylalanine (p-CPA) containing 1.0% arabic gum and 450 mg/kg p-CPA containing 0.1% Tween 80 resulted in a similar drop of the serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) level in the anterior (HA) and posterior (HP) hypothalamus, midbrain (GC) and amygdala (AM). Despite the fact that just the same decrease in the 5-HT turnover occurred in both groups, only in the group treated with p-CPA containing arabic gum a rise in post-carbachol emotional-defensive response was observed. The level of dopamine (DA) did not change in both groups. The level of noradrenaline (NA) in the p-CPA with arabic gum treated group was not subject to any change, whereas in the p-CPA with Tween 80 treated group its considerable decrease was noticed, though no essential statistical differences appeared: in HA by 26%, in HP by 38%, in GC by 23% and in AM by 62% as compared to control group (NaCl treated) and in HA by 35%, in HP by 41%, in GC by 33% and in AM by 50% as compared to the p-CPA with arabic gum treated group. In all groups of animals there occurred neither spontaneous aggressive behavior nor changes in spontaneous motility. The results obtained make it clear that Tween 80 exerts its effect both on biochemical and behavioral processes. Thus, it should be acknowledged that using sorbitans in the investigation of brain mechanisms is inadvisable.

INTRODUCTION

In our previous study (9) we pointed out that i.p. injection of 0.1% saline solution of Tween 80 resulted in a decrease of post-carbachol growling response in cats, while at the same time, there occurred a rise in the 5-HT turnover in the emotional structures of the brain, i.e. hypothalamus, midbrain and amygdala. Consequently, the obtained results indicate that Tween 80 acts per se and therefore we raised a question whether it was advisable to use sorbitans while preparing suspensions of chemical compounds solutions used in the investigations of brain mechanisms. In looking through the literature we have not come across any objections of this kind, and as our results have no reference to any other research, we have found it relevant to perform further experiments in order to obtain some additional information supporting our view. The problem is of the utmost importance, because sorbitans are often, though not generally, used in various neurochemical and neuropharmacological investigations. In the present study another scientific approach was applied — Tween 80 was added to the suspension of p-CPA solution, the compound which brings about a definite attenuating effect on the 5-HT synthesis (3). Also, it is a well established fact that a decrease in the 5-HT level in the brain result in a rise in general emotional arousal, causes an increase in aggressive behavior and intensification of various types of emotiolal-defensive responses.

Additionally, the experiments in this study were designed to investigate if and to what degree an attenuation of the 5-HT system activity affecties the level of cats motor activity.

MATERIAL AND METHODS

Subjects and surgery

The experiments were carried out on 15 cats of both sexes. All cats had two cannulas chronically bilaterally implanted in the anterio-medial hypothalamus according to the stereotaxic coordinates of Snider and Niemer's atlas (10): A = 13.0, L = 1.5, H = −3.0. The details of surgery and microinjection procedure were described earlier (6).

Drugs

Carbachol (carbachol puriss, Koch-Light) was dissolved in a 0.9% NaCl solution and injected bilaterally 4 μg/1 μl into each part of hypothalamus, p-Chlorophenylalanine (DL-p-Chlorophenylalanine, Sigma) was

prepared in a concentration of 450 mg/5 cm^3 of 0.9% NaCl solution containing 1.0% of arabic gum and 450 mg/5 cm^3 of 0.9% NaCl solution containing 0.1% of Tween 80 (polyoxyethylenesorbitan monooleate, Sigma). p-CPA was given in a single dose of 450 mg/kg i.p. The control group was treated with a 0.9% NaCl solution in the same way in a volume 5 cm^3/kg.

Experimental procedure

Recording of the spontaneous motility. Spontaneous motility in cats was measured by the open-field method in the experimental chamber, whose dimensions were 100 × 100 × 70 cm. The floor of the chamber consisted of 100 plexiglass panels measuring 9 × 9 cm each, which were independent recording units; a moving animal pressed them, which was being recorded by the automatic counting system and then printed. The experimental chamber was placed in a separate, partly noise-attenuating enclosure. In the adjoining room the experimentator was attending to the recording system as well as observing the behavior of the animals on a television monitor. The animals' motility was registered twice within 30 min at 7 day intervals, and means values from these two experiments established the control level for the data obtained 36 h following either p-CPA or NaCl injection. Recordings of motility were carried out at 8 o'clock in the morning. This being done, the cats were immediately carried into another experimental chamber, where emotional-defensive responses evoked by intrahypothalamic carbachol injection were recorded.

Recording of the post-carbachol growling response. The intensity of the emotional-defensive behavior evoked by carbachol injections into the hypothalamus was evaluated by recording the latency period of growling response (L), the total number of growls (N), the total number of their duration (T) and the total time duration of vocalization (D) (for details see 1,6). The response was considered completed if a growl was not followed by another within 3 min. All the cats were tested twice at 7 day intervals for growling response and mean values from these two experiments established the control level for the data obtained 36 h following p-CPA or NaCl injection. The animals were then divided into three groups:

Group I ($n = 5$). The cats were injected i.p. with 0.9% NaCl solution in a volume of 5 cm^3/kg, and intensity of the carbachol-induced growling response and spontaneous motility was measured 36 h after the saline injection.

Group II ($n = 5$) like Group I, but p-CPA in a dose of 450 mg/kg containing 1.0% of arabic gum was injected i.p.

Group III ($n = 5$) like Group I, but p-CPA in a dose of 450 mg/kg containing 0.1% of Tween 80 was injected i.p.

Sixty h after p-CPA (or NaCl) injections all cats were killed by decapitation; their brains were rapidly removed and four structures, i.e. the anterior hypothalamus (HA), the posterior hypothalamus (HP), the midbrain central grey matter (GC) and the amygdala (AM) were separated with dissection and kept frozen (for details see 8). Concentrations of noradrenaline (NA), dopamine (DA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in HA, HP, GC and AM were determined spectrofluorometrically according to the method of Earlier and Leonard (2).

Statistics

Results were analyzed by ANOVA (mixed design) followed by Duncan's multiple comparison test.

RESULTS

Biochemical results

ANOVA demonstrated statistically significant differences in the contents of 5-HT and 5-HIAA between groups, which were $F_{2/12} = 4.14$, $p < 0.042$ for 5-HT and $F_{2/12} = 22.53$, $p < 0.001$ for 5-HIAA. Some further and more detailed analysis by means of Duncan's test showed that the level of 5-HT was lower in Group II vs. Group I ($p < 0.05$) and Group III vs. Group I ($p < 0.05$) and there were no differences in Group III vs. Group II. The level of 5-HIAA was lower in Group II vs. Group I ($p < 0.001$) and in Group III vs. Group I ($p < 0.001$); there were no differences in Group III vs. Group II, either (Table I). No significant differences occurred in the contents of 5-HT and 5-HIAA in particular brain structures. Moreover, there were no significant interactions between brain structures and groups.

No significant differences in the contents of NA and DA between particular groups were observed, however, it was demonstrated that statistically significant differences existed between particular brain structures, which were $F_{3/36} = 27.71$, $p < 0.001$ for NA and $F_{3/36} = 27.56$, $p < 0.001$ for DA. No interactions occurred between brain structures and groups.

Despite the fact that there were no statistically significant differences in the contents of NA in particular experimental groups, it should

TABLE I

Regional brain concentration of NA, DA, 5-HT and 5-HIAA in Group I — i.p. 0.9% NaCl solution injected, Group II — i.p. p-CPA containing 1.0% arabic gum injected and Group III — i.p. p-CPA containing Tween 80 injected

Group (treatment)	Brain region	Amine content in μg/g wet tissue (mean ± SEM)			
		NA	DA	5-HT	5-HIAA
I-NaCl		1.386±0.244	2.602±0.588	0.499±0.170	0.986±0.141
II-p-CPA+ arabic gum		1.574±0.292	3.532±1.524	0.190±0.045 $p < 0.05$	0.177±0.056 $p < 0.001$
I-NaCl	HA	1.386±0.244	2.602±0.588	0.499±0.170	0.986±0.141
III-p-CPA +Tween 80		1.026±0.244	2.106±0.296	0.268±0.129 $p < 0.05$	0.344±0.178 $p < 0.001$
II-p-CPA +arabic gum		1.574±0.292	3.532±1.524	0.190±0.045	0.177±0.056
III-p-CPA +Tween80		1.026±0.244	2.106±0.296	0.268±0.129	0.344±0.178
I-NaCl		0.883±0.118	1.627±0.260	0.385±0.148	0.871±0.064
II-p-CPA+ arabic gum		0.928±0.196	1.449±0.245	0.142±0.065 $p < 0.05$	0.216±0.033 $p < 0.001$
I-NaCl	HP	0.883±0.118	1.627±0.260	0.385±0.148	0.871±0.064
III-p-CPA +Tween 80		0.543 ±0.144	1.618 ±0.096	0.117 ±0.060 $p < 0.05$	0.111 ±0.019 $p < 0.001$
II-p-CPA +arabic gum		0.928±0.196	1.449±0.245	0.142±0.065	0.216±0.033
III-p-CPA +Tween 80		0.543±0.144	1.618±0.096	0.117±0.060	0.111±0.019
I-NaCl		0.509±0.154	1.412±0.544	0.593±0.200	0.819±0.099
II-p-CPA+ arabic gum		0.581±0.174	1.280±0.387	0.211±0.130 $p < 0.05$	0.297±0.182 $p < 0.001$
I-NaCl		0.509±0.154	1.412±0.544	0.593±0.200	0.819±0.099
III-p-CPA +Tween 80	GC	0.390±0.092	1.234±0.148	0.113±0.046 $p < 0.05$	0.103±0.022 $p < 0.001$
II-p-CPA+ arabic gum		0.581±0.174	1.280±0.387	0.211±0.130	0.297±0.182
III-p-CPA +Tween 80		0.390±0.092	1.234±0.148	0.113±0.046	0.103±0.022
I-NaCl		1.008±0.237	4.817±0.584	0.544±0.155	0.876±0.064
II-p-CPA+ arabic gum		0.759±0.104	4.187±1.054	0.136±0.036 $p < 0.05$	0.394±0.067 $p < 0.001$
I-NaCl		1.008±0.237	4.817±0.584	0.544±0.155	0.876±0.064
III-p-CPA +Tween 80	AM	0.376±0.104	4.566±0.366	0.110±0.029 $p < 0.05$	0.340±0.051 $p < 0.001$
II-p-CPA+ arabic gum		0.759±0.104	4.187±1.054	0.136±0.036	0.394±0.067
III-p-CPA +Tween 80		0.376±0.104	4.566±0.366	0.110±0.029	0.340±0.051

Statistical significance: Duncan's test

be pointed out that in Group III (p-CPA with Tween 80 treated cats) there occurred a great percentage lowering of the NA level in HA by 26%, in HP by 38%, in GC by 23% and in AM by 62% as compared to Group I (NaCl treated animals) and in HA by 35%, in HP by 41%, in GC by 33% and in AM by 50% as compared to Group II (p-CPA with arabic gum treated animals).

Spontaneous motility

ANOVA demonstrated that there were no significant differences in motility either between groups or repeated measures before and after treatment. No significant interactions occurred between treatment and groups.

Post-carbachol growling response

ANOVA showed that there were no significant differences as regards all the measured parameters of post-carbachol growling response, i.e. latency period, number of growls, time of their duration and time duration of vocalization both between groups and treatment. The only significant interactions between treatment and groups occurred with respect to the number of growls: $F_{2/12} = 4.52$, $p < 0.034$. By means of Duncan's test for interactions it was demonstrated that there was a significant increase in the number of growls only in Group II 36 h following the administration of p-CPA containing 1.0% arabic gum as compared to control level (before treatment) ($p < 0.05$) (Fig. 1).

After the administration of p-CPA, which causes a decrease in the serotonergic system activity, no cat out of 10 displayed any spontaneous aggressive behavior.

DISCUSSION

The results obtained in the current investigation provide substantial support for the view-presented in our previous study (9) that it is inadvisable to use sorbitans in the investigation of brain mechanisms. Further data, illustrating how significant an effect on behavioral as well as neurochemical processes Tween 80 exerts, were obtained. In the cats of Group III, 36 h following the injection of p-CPA containing 0.1% Tween 80, no changes in the intensity of post-carbachol emotional-defensive response were observed. In Group II however the same dose of p-CPA containing 1.0% arabic gum caused at the same time an increase in post-carbachol defensive response, illustrated by a statistically significant rise in the number of growls. Moreover, if should be pointed out that in both

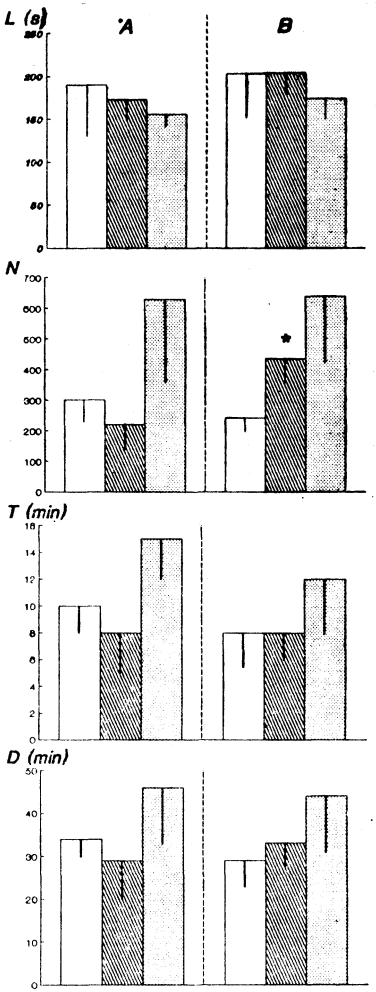

Fig. 1. The effect of i.p. 0.9% NaCl solution (white bars), 450 mg/kg p-CPA containing 1.0% of arabic gum (dashed bars) and 450 mg/kg p-CPA containing 0.1% of Tween 80 (doted bars) administration on carbachol-induced growling response. Mean latent period of growling response (L), mean number of growls (N), mean time of their duration (T) and mean time duration of vocalization (D) ± SEM. Control level before NaCl or p-CPA treatment (A) and 36 h after treatment (B). $*p < 0.05$ as compared to the control data, Duncan's test.

groups p-CPA caused a similar fall in the 5-HT and 5-HIAA level in all the investigated brain structures. This evidence speaks strongly for the fact that it was due to the action of Tween 80 that an increase in the emotional-defensive behavior in Group III was not observed. Numerous data obtained in investigations carried out by different authors on various animals and models of emotional-defensive responses provide a reliable and explicit support for the interpretation that as a result of a decrease in the 5-HT system activity an increase in the emotional-defensive behavior occurs (ref. 4, 11). We observed much the same dependence in our earlier investigations (8) in the applied by us model of postcarbachol emotional-defensive response in cats. Further evidence supporting the

point that Tween 80 exerts an effect on the central mechanisms is the fact that in Group III (p-CPA containing Tween 80) in all the examined structures a considerable lowering of the NA level occurred, which was not observed in Group II (p-CPA containing arabic gum) as well as in earlier experiments (8) in which p-CPA suspension was prepared in a 0.9% NaCl solution. Also, some other authors using p-CPA suspension containing Tween 80 in their investigations noticed that the level of NA decreased by 20 - 40% (3, 5). However, this fact was not related to action of the sorbitan.

The results obtained in the present experiments demonstrated that in cats, unlike in rats, neither spontaneous aggressive behavior nor an increase in motility following the attenuation of the 5-HT system function occurred. The present results are congruent with the earlier findings obtained after 5-HT depletion by means of p-CPA (8) as well as after chemical lesions of 5-HT neurons in the dorsal raphe nucleus (7). It is likely that we have to do here with certain specific species characteristics.

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