

IS IT ADVISABLE TO USE SORBITANS IN THE INVESTIGATION OF THE BRAIN MECHANISMS?

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Abstract. Injection of 0.1% saline solution of Tween 80 i.p. ($3 \times 3 \text{ cm}^3/\text{kg}/12 \text{ h}$) in the cat decreased the carbachol-induced growling response 36 h after the last injection. The content of 5-hydroxyindoleacetic acid (5-HIAA) increased in the hypothalamus, midbrain and amygdala, which indicates the increase in serotonin (5-HT) turnover and the 5-HT system activity. The results indicate that Tween 80 causes per se both behavioral and neurochemical changes. Therefore, it is inadvisable to add sorbitans to the solutions used in the investigation of the brain mechanisms.

INTRODUCTION

Sorbitans have been widely applied in pharmacology for drug production, where their use seems to be justified and sometimes simply necessary. Some appropriate investigation precedes the promess of preparing drugs containing sorbitans, since their presence may exert an influence on absorption, durability and biological activity of the drug, affecting at the same time its curative effect considerably. Therefore, sorbitans are not pharmacologically neutral compounds.

In scientific investigations sorbitans are also sometimes used for solubilization and to prepare suspensions (1, 4-7, 12, 13). Having observed that it is not unlikely that Tween 80 added to a solvent may affect central mechanisms of emotinal-defensive response we undertook the

present investigation to ascertain whether the suspicions are not without reason. We have not come across any information on this subject in the scientific publications.

MATERIAL AND METHODS

Subjects and surgery

The experiment was performed on 21 cats of both sexes. All cats had chronically bilaterally implanted cannulas to the antero-medial hypothalamus according to the stereotaxic coordinates of Snider and Niemer's atlas (11): A = 13.0, L = 1.5, H = -3.0. The details of surgery and microinjections procedure were described earlier (9).

Chemical compounds

Carbachol (carbachol puriss., Koch-Light) was dissolved in a 0.9% NaCl solution and injected bilaterally, 4 $\mu\text{g}/1 \mu\text{l}$ into each part of the hypothalamus. Tween 80 (polyoxyethylenesorbitan monooleate, Sigma) as 0.1% saline solution was used for i.p. injections.

Experimental procedure

The intensity of the emotional-defensive behavior evoked by bilateral intrahypothalamic injections of carbachol was evaluated by recording the latency period of growling (L), the total number of growls (N), the total time of their duration (T), and the total time of vocalization (D) (for details see 2). The response was considered completed if a growl was not followed by another within 3 min. All the cats were tested one time for the growling response, and it was an initial (control) level of the carbachol-induced emotional-defensive behavior. The animals were then divided into three groups:

Group I ($n = 5$). Cats were not exposed to any examination before being sacrificed by decapitation, i. e. received no treatment.

Group II ($n = 6$). Cats were injected i.p. with 0.9% NaCl solution in a volume of 3 cm^3/kg three times every 12 h, and intensity of the carbachol-induced growling response was measured on the 12 h and 36 h after NaCl injections.

Group III ($n = 10$) as Group II, but 0.1% saline solution of Tween 80 i.p. was injected.

Six h after the last test for carbachol-induced growling response all cats were killed by decapitation; their brains were rapidly removed and four structures, i. e. the anterior hypothalamus (HA) (frontal planes A 11.0-15.0), the posterior hypothalamus (HP) (frontal planes A 7.0-10.0),

the midbrain central grey matter (GC) (frontal planes A 1.0-5.0), and the amygdala (AM) including parts of nuclei corticomediales and basolateralis (frontal planes A 11.0-14.0) were separated by dissection and kept frozen. 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 (3).

We applied identical experimental procedure as in the previous investigation of i.p. p-chlorophenylalanine injection (10) in order to have an accurate point of reference to the previous experiments and to use from them the group of animals injected i.p. with 0.9% NaCl alone.

Statistics

Biochemical results were elaborated by the Student *t* test for unrelated data; significance of changes in the growling response was evaluated by the Student *t* test for paired data.

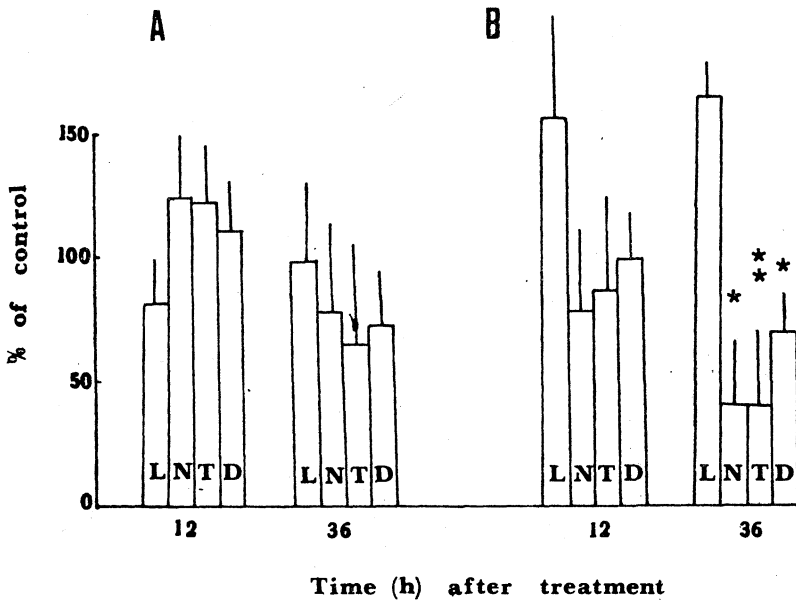


Fig. 1. The effect of i.p. 0.9% NaCl (A) and 0.1% saline solution of Tween 80 (B) administration on growling response evoked by intrahypothalamic carbachol injections. Mean latent period of growling response (L), mean number of growls (N), mean time duration of growling (T) and mean time duration of vocalization (D) \pm SEM. All values are expressed as a percentage of initial level before i.p. NaCl or Tween 80 treatment. Differences from initial values: * $P < 0.02$, ** $P < 0.01$ (paired *t* statistics).

TABLE I

Regional brain concentrations of NA, DA, 5-HT and 5-HIAA in Group I — no treatment, Group II — i.p. 0.9% NaCl injected and Group III — i.p. 0.1% saline solution of Tween 80 injected

Group	Brain region	Amine content in $\mu\text{g/l g}$ wet tissue (mean \pm SEM)								
		NA	%	DA	%	5-HT	%	5-HIAA	%	
I No treatment	HA	0.705 \pm 0.073		1.135 \pm 0.141		0.652 \pm 0.047		0.320 \pm 0.036		
II 0.9% NaCl		0.754 \pm 0.104		1.038 \pm 0.164		0.671 \pm 0.050		0.285 \pm 0.010		
II vs. I		NS	107	NS	91	NS	103	NS	89	
III 0.1% Tween 80		0.694 \pm 0.070		1.601 \pm 0.295		0.671 \pm 0.107		0.432 \pm 0.026		
III vs. I		NS	98	NS	141	NS	103	$p < 0.05$	135	
III vs. II		NS	92	NS	154	NS	100	$p < 0.001$	151	
I No treatment		HP	0.449 \pm 0.096		0.595 \pm 0.069		0.838 \pm 0.048		0.305 \pm 0.027	
II 0.9% NaCl			0.509 \pm 0.090		0.644 \pm 0.091		0.826 \pm 0.064		0.345 \pm 0.025	
II vs. I			NS	113	NS	108	NS	98	NS	113
III 0.1% Tween 80			0.514 \pm 0.052		1.059 \pm 0.174		0.767 \pm 0.117		0.513 \pm 0.039	
III vs. I	NS		114	NS	178	NS	91	$p < 0.01$	168	
III vs. II	NS		101	NS	164	NS	93	$p < 0.01$	149	
I No treatment	GC		0.348 \pm 0.023		0.498 \pm 0.033		0.848 \pm 0.046		0.367 \pm 0.015	
II 0.9% NaCl			0.336 \pm 0.042		0.559 \pm 0.040		0.883 \pm 0.018		0.358 \pm 0.032	
II vs. I			NS	96	NS	112	NS	104	NS	97
III 0.1% Tween 80			0.366 \pm 0.029		0.673 \pm 0.126		0.921 \pm 0.094		0.544 \pm 0.052	
III vs. I		NS	105	NS	135	NS	109	$p < 0.05$	148	
III vs. II		NS	109	NS	120	NS	104	$p < 0.05$	152	
I No treatment		AM	0.283 \pm 0.052		1.618 \pm 0.140		0.442 \pm 0.043		0.275 \pm 0.042	
II 0.9% NaCl			0.304 \pm 0.048		1.774 \pm 0.158		0.482 \pm 0.073		0.289 \pm 0.037	
II vs. I			NS	107	NS	110	NS	109	NS	105
III 0.1% Tween 80			0.405 \pm 0.035		2.129 \pm 0.234		0.592 \pm 0.096		0.468 \pm 0.038	
III vs. I	NS		143	NS	131	NS	134	$p < 0.01$	170	
III vs. II	NS		133	NS	120	NS	123	$p < 0.01$	162	

RESULTS

Thirty-six h after i.p. 0.1% saline solution of Tween 80 injections the number of growls, the time of their duration, and the time of vocalization significantly decreased (Fig. 1B), while no change was observed in i.p. 0.9% NaCl-treated cats (Fig. 1A).

In the cat treated with i.p. 0.1% saline solution of Tween 80 (Group III) the level of 5-HIAA in all investigated brain regions was significantly increased. The change remained identical when Group III was compared with Group I as well as with Group II (Table I).

DISCUSSION

The obtained data indicate explicitly that Tween 80 acts per se causing both behavioral and neurochemical changes. Therefore it must be admitted that it is absolutely inadvisable to add Tween 80 to the solutions used in the investigations of brain mechanisms. One can also suppose that other sorbitans of similar chemical structure will act in more or less the same way.

The activity of Tween 80 in our experiments was demonstrated only in relation to the 5-HT system. Unfortunately, limitations of methodological nature did not allow us to measure metabolites of the NA and DA, which of course restricted the possibility of obtaining information on the functional changes occurring in the NA and DA systems. Taking into consideration the action of sorbitans on membrane processes, one can hardly imagine this action to be selective, referring to 5-HT neurons only.

The results of the present investigation provide one more evidence that the intensity of postcarbachol emotional-defensive response is closely correlated with the 5-HT system activity. In our previous investigation, after chemical lesions of dorsal raphe nuclei (8) as well as after the blocking of 5-HT synthesis by means of p-chlorophenylalanine (10) we observed an increase in the intensity of postcarbachol growling response correlated with the fall of the 5-HT system activity, whereas in the present experiment the situation was exactly opposite, i.e. an increase in the 5-HT system activity was correlated with the drop of intensity of postcarbachol response.

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