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Task-Dependent Development of Tolerance to Scopolamine

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ROSIC, N., D. BOKONJIC AND D. H. OVERSTREET. *Task-dependent development of tolerance to scopolamine.* PHARMAC. BIOCHEM. BEHAV. 13(2) 183–186, 1980.—Rats were chronically treated with once daily injections of either 0.5 mg/kg scopolamine hydrochloride or isotonic saline for 21 days. When spontaneous locomotor activity or acquisition of active avoidance in a two-way shuttle box were measured at 48 hours after the cessation of chronic treatment, no differences were observed between the two chronically treated groups. Tolerance to scopolamine's locomotor stimulatory effects was evident as the increase in locomotor activity following acute treatment was smaller in the group which had been chronically treated with scopolamine. On the other hand, acutely administered scopolamine facilitated the acquisition of active avoidance responding to an equal degree in both chronically treated groups. The reasons which may account for this task-dependent tolerance development to scopolamine are discussed.

Scopolamine Tolerance development Spontaneous locomotor activity
Acquisition of active avoidance responding Task dependent

TOLERANCE development is frequently associated with the chronic administration of drugs. However, it is becoming increasingly apparent that multiple factors are involved in determining the characteristics of tolerance development. These include variables such as type of task, the drug itself, and the schedule of administration, e.g. dose and time [2, 3, 5, 8, 11, 15]. A considerable amount of literature has accumulated on the consequences of chronic administration with amphetamine and there is evidence for tolerance development to some of its effects, slight or no tolerance development to others, and reverse tolerance to still others [3, 4, 10, 15, 16]. Much less attention has been paid to the consequences of chronic administration with scopolamine, an anticholinergic agent which is similar to amphetamine in its acute behavioural effects (see [1]). There is evidence that animals performing an operant task can learn to compensate for the effects of scopolamine [9], but in general there is relatively little information about the characteristics of tolerance development to this agent.

There has also recently been an increased interest in the possibility that alterations in neurotransmitter receptors may be involved in the development of tolerance to drugs (see [12]). However, there have been comparatively few reports which have conclusively demonstrated the involvement of a specific receptor alteration in the development of tolerance to a particular drug. The present set of experiments were designed to characterize the development of tolerance to scopolamine in order to provide the basis for subsequent studies on the possible involvement of receptor alterations in the development of tolerance. In the present paper we report that tolerance development to the stimulatory effects of scopolamine on spontaneous locomotor activity (SMA), but not to its facilitatory effects on acquisition of an active avoidance (AA) response.

METHOD

Animals

The animals were male Wistar rats, approximately 90 days old and weighing between 200–250 g at the beginning of the experiments. They were housed in groups of 7 under conditions of constant temperature and humidity, with free access to water and food.

Apparatus

Spontaneous locomotor activity (SMA) was recorded in two automated Animex motility meters (LKB-Stockholm), whose sensitivity was tuned at 40 μA.

Acquisition of two-way active avoidance (AA) was studied in a series of automatically operated commercial shuttle-boxes and programming-recording units (Ugo Basile, Italy). Boxes measured 48×21×22.5 cm and were used without the central partition.

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TABLE 1

EFFECTS OF SCOPOLAMINE ON SPONTANEOUS LOCOMOTOR ACTIVITY IN RATS CHRONICALLY TREATED WITH SCOPOLAMINE OR ISOTONIC SALINE

Dose of scopolamine acutely administered	Mean activity ± SEM (n)			
	First replication*		Second replication*	
	scopolamine	saline	scopolamine	saline
0 (Baseline)	1222 ± 70 (29)	1231 ± 61 (29)	1001 ± 75 (23)	1183 ± 78 (24)
0.1	1500 ± 104 (8)	1479 ± 232 (8)	1760 ± 137 (5)	2036 ± 368 (5)
0.5	2456 ± 113 (8)	3187 ± 157 (8)†	1921 ± 222 (5)	3451 ± 219 (5)†
1.0	1700 ± 124 (5)	2393 ± 310 (5)†	2502 ± 262 (5)	3443 ± 259 (5)†
2.0	2210 ± 124 (8)	2922 ± 308 (8)†	2440 ± 114 (8)	3438 ± 245 (9)†

*This experiment was carried out twice with separate groups of animals. The baseline measure was taken 48 hr after cessation of chronic scopolamine or saline treatment.

†Siginificantly different, $p<0.05$, from corresponding scopolamine groups (two-tailed t-tests for independent samples).

Administration of Scopolamine

In all experiments rats were treated subcutaneously either with scopolamine or isotonic saline injections. The volume of injection was 1 ml/kg.

During chronic treatment rats were injected once daily with either 0.5 mg/kg scopolamine hydrochloride or isotonic saline for 21 days. All tests for SMA or AA acquisition were conducted at least 48 hours after the last treatment of the chronic regimen.

During acute administration of scopolamine, all rats were tested for SMA or AA acquisition, 30 min after drug administration.

Procedure

SMA. All of the animals were individually placed in the Animex recorder 48 hours after the last injection of scopolamine or saline for the recording of baseline, 30 min period of activity. On the following day, the animals were placed also individually in the Animex immediately after receiving an acute injection of either 0.1, 0.5, 1.0 or 2.0 mg/kg of scopolamine hydrochloride. In order to avoid the influence of the habituation process, a 30 min period of activity was recorded 30 minutes later (or, SMA activity was measured between 30 and 60 minutes after drug treatment). In all acute experiments, rats were treated and used only once. Data are presented as the total amount of activity counts during the 30 min recording session.

Acquisition of AA. A conventional two-way AA schedule was used with trials starting at 30 sec intervals. Each trial began with the turning on of a non-directional light conditioned signal (CS), provided by two 10 W light bulbs at the centre of the ceiling, followed 3 sec later by a 1.5 mA scrambled foot shock (US) administered through a transformer and resistor in series. A crossing response during the CS (avoidance response) terminated the CS and prevented US onset. A response after US onset (escape response) terminated both CS and US. Intertrial crossing was not punished.

The effects of scopolamine on acquisition of AA were studied under two different experimental (behavioural) procedures, massed and spaced trials respectively. In the massed trials condition, the animals were given two sessions each of 100 trials, over two consecutive days. On the first day, all animals were trained without any acute treatment. This was done in order to assess the "floor effect" of AA conditioning (to obtain a baseline level of responding). On the following day, all animals received a second session of 100 trials in the shuttle-boxes; however, half the animals in both groups (scopolamine and saline-treated rats) received an acute injection of 0.5 mg/kg scopolamine, and half an acute injection of isotonic saline 30 min before being placed in the shuttle-box. All experiments carried out on the second day were balanced for assignment to different shuttle-boxes, times of day which animals were tested and baseline obtained on the first day of training. The data are expressed as differences in AA responses between first and second sessions.

In the spaced trials condition, the animals were given daily 50-trial sessions for four consecutive days. Half of the animals received an acute injection of scopolamine (0.5 mg/kg) and half received acute isotonic saline 30 min before each daily session of AA training. The design of experiments was balanced for assignment of rats to shuttle-boxes and time of day. The data are expressed as mean number of total AA responses during all 4 days of training.

To determine whether the facilitatory effects of scopolamine on acquisition of active avoidance were state-dependent, a fifth session of 50 trials was given to some animals. At the appropriate time before this session (30 min), the animals received either the same treatment they had been receiving on the previous four days or a different treatment; i.e. 0.5 mg/kg scopolamine or isotonic saline.

RESULTS

The effects of scopolamine on SMA are summarized in Table 1. No differences in locomotor activity were observed between the two chronically treated groups when SMA was measured at 48 hours after cessation of chronic drug treatment (0 baseline in Table 1).

Following acute administration of scopolamine, SMA was elevated over baseline in both groups. Within group analyses, i.e. looking for statistically significant differences before and after acute scopolamine within each of the dosage level groups, confirmed that SMA was significantly elevated over baseline for each dose of scopolamine in the group chronically treated with saline. However, SMA was not significantly elevated over baseline for the 0.1 mg/kg dose of

TABLE 2

EFFECTS OF SCOPOLAMINE ON ACQUISITION OF MASSED TRIALS ACTIVE AVOIDANCE RESPONDING IN RATS CHRONICALLY TREATED WITH SCOPOLAMINE OR SALINE

	Mean differences in active avoidance responses*	
Acute treatment	Chronic treatment	
	Scopolamine	Saline
Scopolamine	+42.74 ± 7.1 (8)	+41.23 ± 4.2 (10)
Saline	+20.00 ± 4.3 (7)	+20.5 ± 3.8 (10)

For mean differences see Methods.

scopolamine in the group chronically treated with scopolamine.

Although the higher doses of scopolamine significantly increased SMA in both groups, the increase in activity was not as marked in the group that had been chronically treated with scopolamine (Table 1). In fact, these groups were significantly less active than the groups that had been chronically treated with saline at each dose level of acutely administered scopolamine, except the lowest. Thus, tolerance development to the locomotor stimulatory effects of scopolamine is evident.

Animals which were trained under the massed trials condition performed better on the second session, as can be seen in Table 2. It is also apparent, however, that acute administration of scopolamine significantly facilitated acquisition of the active avoidance responses, as the difference scores were much higher for these groups (see Table 2). A final feature of Table 2 is that the facilitation of AA responding by acutely administered scopolamine was similar in both chronically treated groups, i.e., there did not appear to be any tolerance development to scopolamine.

The effects of scopolamine on acquisition of AA over the spaced trials of 50 per day for four consecutive days, summarized in Table 3, lead to similar conclusions. Acutely administered scopolamine significantly increased the number of avoidance responses regardless of whether the animals had been chronically treated with scopolamine or isotonic saline. Again, there was no evidence for tolerance development to scopolamine's facilitatory effects on acquisition of AA.

As can be seen in Table 4, these stimulatory effects of scopolamine were state-dependent. When saline was substituted for scopolamine on the fifth sessions, there was a dramatic decrease in the number of avoidance responses. The degree of decrease was similar for both chronically treated groups. Also evident in this table is the stable high performance of the groups which continued to receive scopolamine.

DISCUSSION

These findings clearly demonstrate that tolerance development to scopolamine is task-dependent. There is a clear-cut, although partial, development of tolerance to the stimulatory effects of scopolamine on SMA (Table 1), but no obvious tolerance development to its facilitatory effects on acquisition of AA (Tables 2 and 3). This task-dependent development of tolerance may be related to the earlier findings of Florio *et al.* [9], who reported that tolerance developed to the effects of scopolamine on go-no go responding, but not to its effects on cortical electrical activity. It is tempting to suggest that the former is related to the tolerance development to the locomotor stimulatory effects of scopolamine whereas the latter may be related to the lack of tolerance development to its facilitatory effects on acquisition of active avoidance reported in the present experiment.

In the present experiments, the argument of behavioural desensitization cannot be used to account for tolerance development to the locomotor stimulatory effects of scopolamine because the behaviour of the animals was not recorded during the period of chronic administration, in contrast to the experiment of Florio *et al.* [9]. The observation that task-dependent tolerance development to scopolamine may occur offers an alternative interpretation of the findings of Florio *et al.* [9]. Only further studies with additional tasks can determine which of these two hypotheses is the more likely.

Previous investigators have suggested that the facilitatory effects of scopolamine and amphetamine on AA responding are closely related to their locomotor stimulatory effects [1]. The present findings, on the other hand, suggest that these two effects of scopolamine are separable. It is important to note, however, that the dose of scopolamine used in the avoidance experiments (0.5 mg/kg) was large enough to produce some locomotor stimulation in the group chronically treated with scopolamine (see Table 1). Whether this small amount of residual locomotor stimulation is sufficient to sub-

TABLE 3

EFFECTS OF SCOPOLAMINE ON ACQUISITION OF SPACED TRIALS ACTIVE AVOIDANCE RESPONDING IN RATS CHRONICALLY TREATED WITH SCOPOLAMINE OR SALINE

	Mean number of total active avoidance responses ± (SEM)			
Acute treatment	First replication*		Second replication*	
	Scopolamine	Saline	Scopolamine	Saline
Saline	56.4 ± 15 (8)	44.2 ± 12 (8)	38.5 ± 8 (17)	42.5 ± 7 (15)
Scopolamine	106.2 ± 10 (8)†	75.9 ± 11 (8)†	94.8 ± 7 (28)†	93.7 ± 4 (27)†

*This experiment was carried out twice with separate groups of animals. The first session occurred 48 hr after cessation of chronic scopolamine or saline treatment.

†Significantly different, $p < 0.05$, from corresponding group acutely treated with saline (two-tailed t-test for independent samples).

TABLE 4

STATE-DEPENDENT EFFECTS OF SCOPOLAMINE ON ACTIVE AVOIDANCE

Treatment*	Mean number of active avoidance responses ± SEM	
	Fourth session	Fifth session
Sc-Sc-Sc	38.7 ± 5.0	35.3 ± 3.8
Sc-Sc-Sal	41.8 ± 7.6	11.0 ± 3.2
Sal-Sc-Sc	27.7 ± 7.0	30.3 ± 7.5
Sal-Sc-Sal	33.7 ± 3.3	11.2 ± 7.0

*The first symbol refers to the period of 21-day chronic treatment with 0.5 mg/kg scopolamine (Sc) of isotonic saline (Sal); the second symbol refers to the acute treatment received 30 min before each of the four training sessions; the third symbol refers to the acute treatment received 30 min before the fifth AA session. Each of these subgroups consisted of 5 rats.

serve the increase in acquisition of AA cannot be determined at this time.

The reasons for the task-dependent development of tolerance to scopolamine are not clear. It is possible that they may be related to separate effects of scopolamine on different neurological subsystems. For example, the locomotor stimulatory effects of scopolamine may be related primarily to its anticholinergic action in the striatum, while its facilitatory effects on acquisition of active avoidance may be related to its anticholinergic action in the hippocampus or cortex. If this hypothesis were correct, then alterations in muscarinic cholinergic receptors may occur in the striatum, but not in the hippocampus or cortex following chronic scopolamine treatment. Studies are underway to examine this possibility.

The task-dependent development of tolerance to scopolamine might also be related to the stimulus properties of this agent. The present findings have confirmed those of others that the effects of scopolamine on acquisition of various responses are state-dependent (Table 4 [1, 2, 13]). More recently, it has been shown that animals can use the stimulus properties of scopolamine and other drugs in performing operant tasks (see [7,14]). Although the reasons are still unclear, it appears that the development of tolerance to the stimulus properties of narcotics is of a smaller magnitude than the development of tolerance to their analgesic effects [6]. This may be related to the smaller doses used to demonstrate stimulus properties. If so, then the dose used in the avoidance studies may have been a suprathreshold dose such that it was still detectable even though some development of tolerance had occurred. More extensive studies are required to address this question.

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