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Facilitation of Conditioned Inhibitory Avoidance by Post-Trial Peripheral Injection of Substance P[1]

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TOMAZ, C. AND J. P. HUSTON. *Facilitation of conditioned inhibitory avoidance by post-trial peripheral injection of substance P.* PHARMACOL BIOCHEM BEHAV 25(2) 469–472, 1986.—Experiments were undertaken to investigate the effects of the neuropeptide Substance P (SP) on performance of a conditioned inhibitory avoidance response in rats. A single-trial inhibitory avoidance task was employed. In Experiment 1 SP was injected IP immediately after the training trial in doses of 0.5, 5, 50, 100, 250 or 500 μg/kg; control animals were injected with diluent vehicle. The group treated with 50 μg SP/kg exhibited better avoidance than the other groups. In Experiment 2 the doses of SP used were 1, 50, 250 μg/kg, and the control animals were injected with vehicle or not injected at all. Only the 50 μg SP/kg treatment group showed significantly better performance. In Experiment 3 50 μg/kg SP or vehicle was injected post-trial immediately or 5 hr after the trial. Only the group in which SP was injected immediately after the training trial showed significantly better performance when tested 24 hr later. This result rules out the possibility that SP exerts its effect by a long lasting proactive action on performance during the testing trial 24 hr later.

Substance P Post-trial Avoidance learning Rat Inhibitory avoidance

THE neuropeptide substance P (SP) has been implicated in learning or memory processing by the results of several studies. For example, it has been shown that inhibitory avoidance learning is sensitive to centrally administered SP, and that the kind of effect obtained depends on the brain site into which it was injected [6–8, 10, 18, 20]. Conditioned avoidance was poorer after post-trial injection of SP into the substantia nigra (SN) and the amygdala, whereas evidence for facilitation of avoidance was found with post-trial administration of SP into the lateral hypothalamus/medial forebrain bundle, the septum, or the nucleus basalis magnocellularis. Similar site-dependent effects in the brain have also been found in terms of SP's properties as a reinforcer [5,21].

There are few reports on the effects of peripheral administration of SP on learning and memory. Hecht *et al.* [4] reported that the IP (intraperitoneal) administration of 250 or 500 μg SP/kg before training did not influence the learning of an active avoidance task in rats. Wetzel and Matthies [23] found that retention of a shock-motivated brightness discrimination in rats was facilitated when SP was administered IP immediately after the training session consisting of 30 trials. Schlesinger *et al.* [16,17] and Pelleymounter *et al.* [13,14] investigated the effects of post-trial subcutaneous and/or intracerebroventricularly administered SP on avoidance conditioning in mice. For example, they found that 1 μg SP/kg given subcutaneously enhanced the retention, whereas higher and lower doses had either less or no effect. The peripheral administration of SP reversed the amnestic effects of cycloheximide and electroconvulsive shock [17].

The aim of the present study was to test the influence of peripheral post-trial administration of SP on learning of the single-trial up-hill inhibitory avoidance task in rats.

METHOD

Subjects and Apparatus

The experiments were performed on 290 male Wistar rats weighing between 210 and 290 g. They were kept on a 12 hr light/12 hr dark cycle in individual plastic cages (38×23×15.5 cm) at an ambient temperature of 20–23°C, with food and water available ad lib.

The experimental apparatus for the up-hill avoidance task has been described previously [9,19]. Briefly, it consisted of a gray plastic box tilted at a 20° angle with 35 cm high walls. Its 50×50 cm floor was covered with wire-mesh netting, which allowed an easy foot hold. Electrical shock (1 mA for

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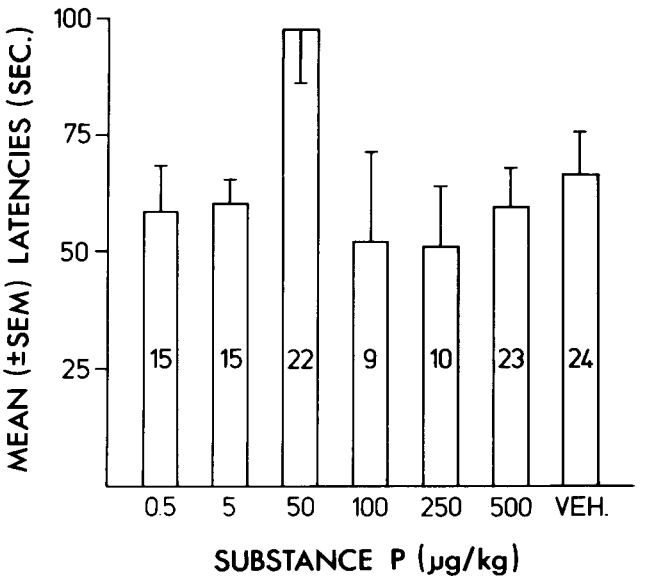

FIG. 1. Effects of SP administered immediately after the training trial on up-hill avoidance performance. During the training trial, the animals received a tail-shock contingent on the up-hill reaction. Retention is expressed as mean (±SEM) latency to turn 90° up-hill measured 24 hr after the training. The numbers in the columns indicate the number of animals used in each treatment group.

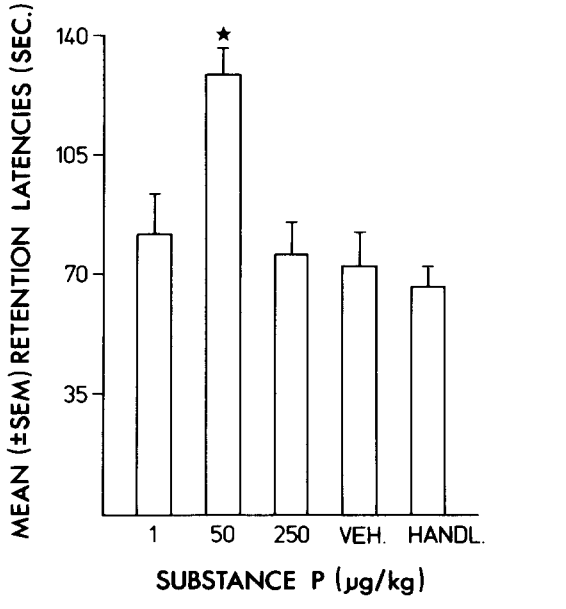

FIG. 2. Effects of SP administered immediately after the training trial on up-hill avoidance. During the training trial the animals received a tail-shock contingent on the up-hill reaction. Retention is expressed as mean (±SEM) latency to turn 90° up-hill measured 24 hr after the training. VEH=vehicle control; HANDL=handling control. ★=Significant difference. The level of significance (5%) was adjusted for four dependent tests to 1.25%; $\alpha\bigstar=\alpha/n$; $\alpha\bigstar=0.05/4=0.0125$.

1 sec) was delivered through a bipolar ring electrode attached to the tail of the animal and connected to a shock generator (Coulbourn Instruments, model E13-16).

Drug Regimen

The SP peptide was dissolved in 0.9% saline containing 0.01 M acetic acid. Control animals received this diluent vehicle. SP was injected IP at doses ranging from 0.5 to 500 μg/kg in a volume of 0.5 ml/100 g body weight. The same volume was used for control animals that received the diluent vehicle. All solutions were blind-coded to eliminate bias.

Experiment 1. In a pilot study, animals received injections of SP in doses of 0.5, 5, 50, 100, 250, or 500 μg/kg, or vehicle. The solutions were injected immediately after the training trial. The number of animals per group ranged from 9–24 (see Fig. 1 for exact number per group).

Experiment 2. A replication of Experiment 1 was performed, except that the animals received injections of SP in doses of 1, 50, 250 μg/kg, or vehicle. Additionally, a handling-control group was included: the animals in this group received the same treatment as the others, but without any injection. Twenty animals were used per group. As in Experiment 1 SP or vehicle was injected immediately after the training trial.

Experiment 3. To test whether the influence of SP in Experiments 1 and 2 could have been due to a long lasting proactive action on performance during the testing trial 24 hr later, SP or vehicle was administered either immediately after the training, as in Experiments 1 and 2, or 5 hr after the training trial. Only the 50 μg SP/kg dose was injected. It was the dose at which SP facilitated performance of the avoidance task in Experiments 1 and 2. Eighteen animals were used per group.

General Training and Testing Procedures

A training trial was begun by fitting the animal with the tail-electrode and then placing it into the center of the 20° inclined box with the nose facing the base. Rats tend to turn around and climb up the incline within 2–10 seconds. They can learn to suppress this response when it is punished with an electrical shock to the tail [9, 19, 20]. An "up-hill response" consists of turning around towards the top of the inclined plane. The up-hill response was defined as completed as soon as the position of the animal changed by at least 90° in either direction as defined by a horizontal plane formed by the base of the tail and one of the forepaws. During the baseline-plus-learning trial the latency for the onset of the up-hill response was measured and the animals received a tail-shock contingent on this response. Immediately thereafter the animals were removed from the testing chamber, disengaged from the tail-electrode, and injected with the solution. In Experiment 3 additional groups were injected 5 hr after the training trial.

Twenty-four hours after this baseline-plus-learning trial the retention of inhibitory avoidance learning was tested in the same test situation, but without tail-shock. A ceiling of 180 sec was imposed on this measure. All animals were trained and tested between 10:00 and 14:00 o'clock.

Statistical Analyses

To compare treatment effects between groups, we used the Mann-Whitney U-Test for large samples with approx-

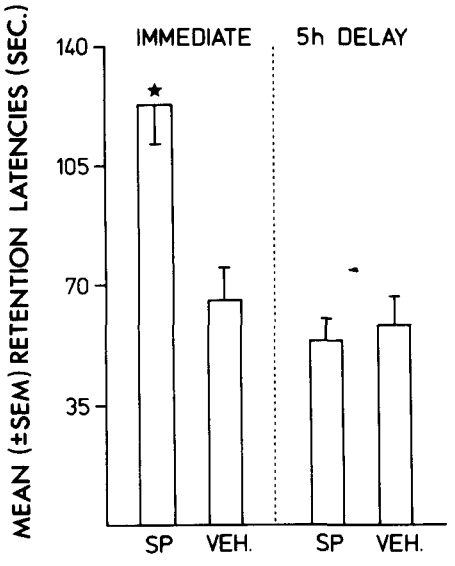

FIG. 3. Effects of post-training administration of SP on avoidance. During the training trial, the animals received a tail-shock contingent on the up-hill reaction. SP 50 μg/kg or VEH (vehicle) was administered either immediately after the training or 5 hr later. Retention is expressed as mean (±SEM) latency to turn 90° measured 24 hr after the training. ★=Significant difference. The level of significance (5%) was adjusted for four dependent tests to 1.25%; $\alpha\bigstar=\alpha/n$; $\alpha\bigstar=0.05/4=0.0125$.

imately normally distributed test statistics [11]. In order to avoid a type I error, which is known to vary with the number of tests conducted [15], the "reduced alpha method" [12] was used to adjust the significance level (5%) for six dependent tests in Experiment 1 to 0.83%, and for four dependent tests in Experiments 2 and 3 to 1.25%.

RESULTS

Experiment 1

SP was injected immediately after the training trial in doses ranging from 0.5 to 500 μg/kg; control animals were injected with diluent vehicle. The results of this pilot study are summarized in Fig. 1.

Only the group that received 50 μg SP/kg showed a trend towards better performance of the up-hill avoidance response. However statistical analyses revealed no differences among the groups appropriate to the level of significance (0.83%) adjusted for six dependent tests.

Experiment 2

The second experiment was essentially a replication of the first, except that the doses of SP utilized were 1, 50, and 250 μg/kg. Control animals were injected with diluent vehicle or not injected at all (handling-control group). Figure 2 summarizes the results.

Comparisons between retention test latencies of the different treatment groups indicated that only the 50 μg SP/kg treatment group showed significantly better conditioned avoidance than the vehicle control group ($p=0.00024$). Thus, the results of this experiment confirm the tendency found in Experiment 1, that SP injected immediately after training at a dose of 50 μg/kg enhances the performance of one-trial up-hill avoidance learning. No significant difference was found between the vehicle injected group and handling control group ($p=0.35745$).

Experiment 3

The purpose of this experiment was to determine whether post-trial injection of SP 5 hr after the training would also have a facilitatory effect on performance as when it was injected immediately after the trial in Experiments 1 and 2 above. The results showed that, unlike immediate post-trial injection, the delayed drug treatment did not facilitate retention test performance. Comparison of the up-hill latencies showed no significant difference between the SP delayed and Vehicle delayed groups ($p=0.42470$). However, an improved performance was observed for the group treated with SP immediately after training, as in Experiments 1 and 2 above. Statistical comparisons revealed significant differences between the SP immediate and Vehicle immediate groups ($p=0.00055$) as well as between the SP immediate and SP delayed groups ($p=0.00007$). The results are summarized in Fig. 3.

DISCUSSION

The main result of the present study was that peripheral post-trial administration of SP improved retention test performance of a single-trial avoidance task in a dose-dependent way. Only 50 μg SP/kg enhanced performance of the up-hill avoidance task; lower and higher doses were ineffective. Additionally, the results of Experiment 3 indicate that the facilitatory effect of SP treatment on performance was not due to a long-lasting proactive influence on performance 24 hr after injection.

These results are consistent with those reported by Wetzel and Matthies [23], who found that 250 μg SP/kg injected IP post-trial, improved discrimination learning in rats. Although the effective dose and learning paradigm were different in the two studies, the results are comparable. In addition, these results are in agreement with those reported by Schlesinger *et al.* [16,17] and Pelleymounter *et al.* [13] who demonstrated a memory enhancing effect of peripheral administration of SP in mice.

Several studies [1,22] have shown that SP can penetrate the blood-brain barrier. Of 18 endogenous peptides tested, SP showed the highest blood brain penetration [1]. Thus, it is possible that the effects on learning obtained with peripheral administration of SP are a result of a central action of the substance. This is all the more likely since direct application of SP in certain parts of the brain has been shown to facilitate learning of avoidance tasks, including the up-hill avoidance task used here [10,20]. If the effects are central effects, it remains to be determined where and in interaction with what neurotransmitters the SP is active.

Regarding the possible site of action in the brain, since the effect of peripheral SP was to facilitate learning, it is tempting to speculate that the central site of action includes the areas (LH, septum, nucleus basalis magnocellularis) where SP injected locally led to a facilitation of avoidance learning [7, 8, 10, 18, 20]. However, it is possible that a direct peripheral effect of SP could be responsible for the post-trial effects.

Schlesinger *et al.* [16] demonstrated that naltrexone, when used in combination with SP, inhibits the effects of SP

on memory processes, which suggests that the facilitatory effects of SP might be mediated via interactions between this peptide and endogenous opioid systems in the brain.

In the present study we observed that only the 50 μg SP/kg dose had a facilitating effect; higher and lower doses were ineffective. Similar inverted U dose-response effects of SP on learning have been obtained by centrally injected [5, 10, 18] as well as by subcutaneously administered SP [16,17].

Patients with senile dementia of the Alzheimer type, show significantly lower cortical SP immunoreactivity than control subjects [2]. Furthermore, injection of SP into the nucleus basalis magnocellularis has been found to facilitate learning of the up-hill avoidance response [10]. There are also observations of abnormally low SP levels in the basal ganglia seen post morten from patients with Huntington's chorea [3]. Given the cumulation of evidence for facilitating effects of peripheral administration of SP, it may be justified to consider possible therapeutic effects of SP in patients with the above mentioned diseases that are known to have profound effects on learning ability and memory.

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