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Long-lasting mnemotropic effect of substance P and its N-terminal fragment (SP1-7) on avoidance learning

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Abstract

We investigated the long-lasting effect of peripheral injection of the neuropeptide substance P (SP) and of some N- or C-terminal SP fragments (SPN and SPC, respectively) on retention test performance of avoidance learning. Male Wistar rats (220 to 280 g) were trained in an inhibitory step-down avoidance task and tested 24 h or 21 days later. Immediately after the training trial rats received an intraperitoneal injection of SP (50 µg/kg), SPN 1-7 (167 µg/kg) or SPC 7-11 (134 µg/kg). Control groups were injected with vehicle or SP 5 h after the training trial. The immediate post-training administration of SP and SPN, but not SPC, facilitated avoidance behavior in rats tested 24 h or 21 days later, i.e., the retention test latencies of the SP and SPN groups were significantly longer ($P<0.05$, Mann-Whitney U-test) during both training-test intervals. These observations suggest that the memory-enhancing effect of SP is long-lasting and that the amino acid sequence responsible for this effect is encoded by its N-terminal part.

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- Avoidance conditioning
- Long-lasting memory

A number of studies indicate that peripheral post-training administration of the neuropeptide substance P (SP) improves retention performance in various memory tasks in a dose- and time-dependent way (1-6). We have demonstrated that intraperitoneal (*ip*) post-training administration of SP to rats enhances memory and that these effects are observed for different tasks with different response requirements and in the absence of explicit punishment (3). Furthermore, we suggested that the memory-enhancing effects of SP are mediated, at least in part, via interactions with the endogenous opioid systems and that the mnemotropic effects of peripherally administered SP are sensitive to the functional integrity of the vagus nerve, suggesting that the vagus nerve may be one pathway by which systemic SP influences memory storage processes in the brain (4). Huston and Hasenöhrl (6) have suggested that the amino acid sequence responsible for mnemonic processing is located in the N-terminus of SP, whereas the reinforcing properties of SP may lie in the C-terminus of the SP molecule.

Although SP and its N-terminal fragments (SPN) produce mnemonic effects, none of the above-mentioned studies investigated the actions of SP on memory processes in animals tested several weeks after learning. As suggested by McGaugh (7), immediate post-training treatments that affect retention tested many days after training indicate that this effect is unlikely to be due to influences acting at the time of the retention test. Fur-

thermore, since the treatment is administered after training, the effects on retention cannot be attributed to influences on acquisition processes. Such findings are interpreted to indicate that the treatment affects retention by modulating memory storage processes. In fact, many experimental manipulations that facilitate memory have been shown to exert long-term effects. For example, experiments dealing with long-term retention show that epinephrine modulates memory in mice tested in a Y-maze discrimination task 24 h, 1 week and 1 month later (8). On this basis, the aim of the present study was to investigate the possible long-term memory effects of SP and its N- and C-terminal fragments (SPN and SPC, respectively) in rats trained in a step-down avoidance task.

The training and testing procedures were as follows: the apparatus consisted of a platform (15 x 11 x 6 cm) fixed in the center of a box with an electrifiable grid floor (50 x 50 x 37 cm). The animals were made familiar with the training apparatus in two daily baseline trials, i.e., each animal was placed on the platform and the latency to step-down on the grid floor with all four paws was measured. During the second trial, when the animal stepped down onto the grid floor, it received 1 sec of a 0.6-mA scrambled footshock. After receiving the footshock, the subject was immediately removed from the apparatus, wrapped in a cloth, and injected intraperitoneally with SP (50 µg/kg), SPN (167 µg/kg), pGlu^{6}-SP6-11 (SPC, 134 µg/kg), or vehicle (V, physiological saline containing 10 mM acetic acid, pH 4.0) in a volume of 0.5 ml/100 g body weight. All injections were blind coded to avoid bias. During the retention test rats were placed on the platform and allowed up to 600 sec to descend from the platform. The animals were divided into the following groups (N = 10 per group), designated according to the treatment and the day when the retention test was performed: a) SP or V immediately post-trial and tested 24 h later, b) SP, SPN, SPC or V immediately post-trial and tested 21 days later. Two separate groups were trained in the same manner, but SP or V was administered 5 h after training.

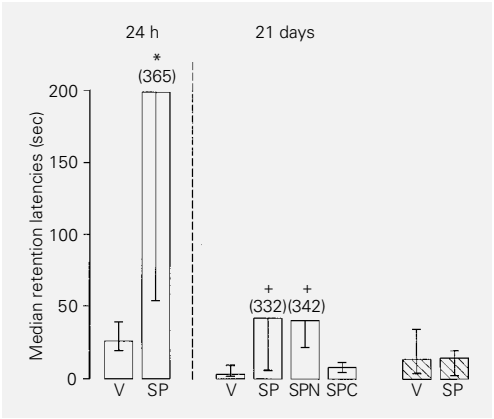

Figure 1 - Effects of peripheral post-trial administration of SP and N- or C-terminal SP fragments (SPN and SPC, respectively) on retention test performance in the step-down avoidance task. Retention is reported as the median (± interquartile range) latency to step-down measured 24 h and 21 days after training. *P<0.05 and $^{+}$P<0.05 compared to control group tested 24 h and 21 days after training, respectively (Mann-Whitney U-test). The level of significance ($^{+}$P<0.05) was adjusted for five dependent tests to 1%; $\alpha = \alpha/n$; 0.005/5 = 0.001. □, Immediate injection; ▨, 5-h delay before injection. The numbers above the columns indicate the highest latency observed in the groups. V, Vehicle.

The results are presented in Figure 1. Comparisons between baseline and retention values indicated that all groups learned the task (P<0.05, Wilcoxon rank sum test, data not shown). The groups receiving SP immediately post-trial showed a significantly better performance than their respective control groups (P<0.05, Mann-Whitney U-test). Animals receiving SP or SPN and tested 21 days later showed performance similar to that of the vehicle control group tested 24 h later. SPC did not affect the retention test and, unlike immediate post-trial injection, the 5-h delayed SP administration did not alter retention performance.

The present results confirm many reports (1-6) suggesting that post-training administration of the neuropeptide SP (50 µg/kg) improves memory. Rats injected with SP or SPN and tested 24 h or 21 days after the original learning showed a better retention performance than the control group. Control animals tested 21 days after the original learning did not show memory in the avoidance task. The saving scores (step-down latencies) of rats injected with SP and SPN, but not SPC, and tested 21 days after the learning trial were similar to those observed

for rats injected with vehicle and tested 24 h later. This result suggests that treatment with SP and SPN produces a long-lasting mnemotropic effect that protects against the natural forgetting, as observed for control animals 21 days after one-trial step-down inhibitory avoidance training. SP injected 5 h after training did not affect retention performance. These results indicate that SP and SPN given immediately after the training trial produce memory-enhancing effects not only in animals tested 24 h later, but also in those tested 21 days later, suggesting long-lasting memory effects. Huston and Hasenöhrl (6) observed that SP produces effects on memory and reinforcement, while the SP fragments exert specific actions when administered to animals tested in both paradigms. SPN facilitates avoidance learning whereas SPC induces place preference, indicating that memory-enhancing and reinforcement effects are encoded by different SP sequences, with the N-terminal 'non-tachycinin sequence' SP1-7 but not the C-terminal sequence being responsible for the memory-promoting effects . This hypothesis is supported by our results, since memory retention is only affected by peripheral injections of SPN.

The results obtained with post-trial injection of SP 5 h after learning showed that the delayed drug treatment did not facilitate retention test performance and ruled out the possibility that SP or SPN exerted its effect by a long-lasting proactive action on performance during the testing trial.

The main finding of the present study is that immediate post-training administration of SP and SPN enhanced retention for at least 21 days after the original training. Such a long-term effect, as originally proposed by McGaugh (7), indicates that memory processes *per se* are affected by the treatment. Neurochemical studies have shown similar long-lasting effects. For example, peripheral as well as intracerebral injection of SP causes a steady and long-lasting increase in extracellular dopamine levels (9). Additionally, increasing evidence supports a role for SP as a neurotrophic and neuroprotector substance (10). Taken together, this evidence suggests that the administration of the neuropeptide SP may alter the physiological state of neuronal systems. However, no functional role for endogenous SP in learning and neuroplasticity has been reported and the site and the mechanism of SP action in these processes are unknown. Pharmacological experiments using high-affinity receptor agonists and antagonists could provide useful information.

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