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Differential Effects on Learning by Ventromedial vs Lateral Hypothalamic Posttrial Injection of Substance P

URSULA STAUBLI AND JOSEPH P. HUSTON

Institute of Pyschology III, University of Düsseldorf, Universitätsstrasse 1, D-4000 Düsseldorf, F.R.G., and Institute of Pharmacology, University of Zürich, Zürich, Switzerland

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STAUBLI, U. AND J. P. HUSTON. *Differential effects on learning by ventromedial vs. lateral hypothalamic posttrial injection of substance P.* PHARMAC. BIOCHEM. BEHAV. **10**(5)783–786, 1979.—The effects of post-trial injection of substance P (SP) into the lateral hypothalamus (LH) and the ventromedial hypothalamus (VMH) on passive avoidance learning was studied in rats. In the VMH, 50 ng and 500 ng SP influenced neither learning of a step-down avoidance nor of an alcove avoidance response. In contrast to these findings, 500 ng SP injected into the LH significantly enhanced retention of the alcove avoidance task. Similarly, in the step-down avoidance experiment, learning was strongly facilitated by posttrial injection of 50 ng as well as 500 ng SP into the LH. These results, together with our previous data showing amnesia with posttrial injection of SP into amygdala and substantia nigra, suggest that exogenously applied SP influences the activity of those brain regions shown to contain high densities of SP-positive nerve terminals. Interestingly, the effects of posttrial SP injection parallel the effects of post-trial electrical brain stimulation on passive avoidance learning. Hence, posttrial SP retroactively facilitates or impairs learning depending on where in the brain it is injected.

Substance P Ventromedial hypothalamus Lateral hypothalamus Posttrial Memory Learning Facilitation

SEVERAL studies have revealed an uneven distribution of the peptide substance P (SP) in the rat brain [3, 12, 20]. This raises the possibility that SP may have a more important role in some parts of the brain than in others. SP is found in especially large amounts in the reticular part of the substantia nigra, in the entopeduncular nucleus and within the amygdaloid complex. High levels have also been reported in hypothalamic nuclei [1, 3, 5, 15]. We have found that posttrial injection of SP into the substantia nigra [11] as well as the medial amygdaloid nucleus [10] causes retrograde amnesia for a step-down avoidance task, which indicates an interaction of SP with learning and memory processes. Notably, the results obtained with posttrial SP injection into the substantia nigra and the amygdala parallel the amnestic effects of electrical stimulation in these brain regions [2, 6, 7, 13, 21, 24].

The hypothalamus was selected as a further site where posttrial SP injection possibly influences learning, for posttrial electrical stimulation of the lateral hypothalamus (LH) was repeatedly shown to facilitate learning [9]. The ventromedial hypothalamus (VMH) was also chosen for posttrial injection of SP, since traditionally, the LH and VMH were considered as two opposing brain "centers." Results of diverse experiments suggest a reciprocal interaction of these two hypothalamic sites [8]. To mention some examples: Lesion of the LH leads to severe aphagia and adipsia, whereas destruction of the VMH causes hyperphagia: electrical stimulation of the LH induces eating in rats, whereas the same stimulation applied to the VMH causes a hungry animal to stop eating. Furthermore, self-stimulation in the LH is readily obtained with high response-rates, whereas self-stimulation in the VMH is unreliable and animals even work to avoid electrical stimulation in the VMH.

Both the LH and VMH were reported by Brownstein *et al.* [3] to contain relatively high levels of substance P. (The present studies were completed prior to publication of the highly relevant finding [17] that unlike the LH - medial forebrain bundle area, the VMH is devoid of SP terminals, although it contains SP cell bodies and single fibers.)

In summary, the purpose of the present experiments was (a) to examine whether SP injected into the hypothalamus has any influence on memory processing and (b) to investigate possible differential effects of SP application in the lateral vs ventromedial hypothalamus on passive avoidance learning.

METHOD

Surgery

Male Sprague-Dawley albino rats, weighing between 280 and 300 g at the time of surgery, were used in the experiment. The animals were sterotaxically implanted under bar-

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biturate anesthesia into the right hemisphere. Using the coordinate system of Pellegrino and Cushman [19] a stainless-steel guide cannula (ga 22, Hamilton Co. Inc.) was inserted either at the level of the lateral hypothalamus (LH) or of the ventromedial hypothalamus (VMH). Implantation was performed at four different sites, i.e. half of the animals with injection sites in the LH were implanted anterior in the LH, the other half more posterior. Similarly, for the VMH a posterior and anterior site was chosen for cannula insertion. The exact coordinates for the LH were AP:6.2 mm, L:1.8 mm, DV:−2.0 mm and AP:4.6 mm, L:1.8 mm, DV:−3.0 mm. For the VMH implantation was performed at AP:6.0 mm, L:0.9 mm, DV:−2.9 mm, and AP:5.4 mm, L:0.7 mm, DV:3.5 mm. These coordinates define the place where the injections were administered, whereas the guide cannula itself was fixed 2 mm above the final injection site. The reason for this procedure was to prevent the tissue around the injection site from being destroyed by the relatively large guide cannula. After fixation of the cannula a stainless-steel mandrel (ga 28, Hamilton Co. Inc.) was inserted into the guide cannula in such a way that it protruded approximately 0.3 mm beyond its tip. The needle was slightly bent in the middle to keep a tight fit, and bent over the upper end of the guide cannula to serve as a permanent plug during intertrial intervals.

Animals which served as sham-operated controls were prepared in exactly the same way as the experimental animals, except that no guide cannula was lowered into the brain.

After surgery and throughout the course of the experiments the animals were individually housed in transparent plastic home cages with unlimited access to food and water. They were maintained on a 12 hr light-dark cycle (7.00 a.m. on – 7.00 p.m. off). All training and testing procedures were performed between 1.00 and 7.00 p.m.

Injection Procedure

For the injection of substance P (SP) or saline into the hypothalamic loci defined above, a stainless-steel injection cannula (ga 28, Hamilton Co. Inc.), which protruded 2 mm below the bottom of the guide cannula, was used. The injection cannula was connected to a 10 μl Hamilton syringe via a polyethylene tube (pp 10, Laubscher and Co. AG, Basel). Microinjections of SP (50 ng and 500 ng) were made in a volume of 0.5μl saline and lasted 30 sec. Control injections of 0.5 μl saline were performed in exactly the same way. In order to ensure optimal infiltration into the tissue the injection cannula was kept in place for an additional 30 sec.

Before starting with the experiments proper a sham injection was performed in all animals by simply inserting an injection cannula. This procedure was done in order to attenuate in the following learning trials with posttrial drug injection a possible additional effect of the lesion caused by the injection needle itself.

Experimental Apparatus

Two one-trial passive avoidance tasks were used: The alcove avoidance task and the step-down avoidance task. (a) The apparatus of the alcove avoidance task consisted of a small black covered box (21×13×16 cm) that was connected to a large grey box (50×50×37 cm) via a small opening (13×16 cm). The large box had no cover and was therefore illuminated by room lighting, in contrast to the small box (alcove). The floor of both boxes consisted of electrifiable grid-bars. (b) The apparatus of the step-down avoidance task consisted of a round platform (14 cm in diameter and 3 cm high) that was fixed in the center of an electrifiable grid-floor of a rectangular box (50×50×37 cm).

Training and Testing Procedures

Alcove avoidance task. Pretraining trials consisted of placing the animal into the large box for 5 min. The time the animal spent in the large and small boxes as well as the time of the first entry into the small box was recorded. Pretraining was considered sufficient and was stopped when the animal spent at least 80% of the total time in the small alcove chamber and entered it within the first 20 sec. This was the case for all animals after three pretraining trials (one per day).

On the following day each rat was directly put into the alcove chamber, which was now separated from the large box by a black sliding door. There a 3 sec duration scrambled footshock of 1 mA was delivered, upon which the animal was immediately removed from the training apparatus. Thirty sec after delivery of the footshock the animal received a posttrial injection of SP or saline according to the injection procedures described above. There were four experimental SP groups, namely a high dosage (500 ng) group for each LH cannula placement (n=14) and VMH cannula placement (n=15), as well as a low dosage (50 ng) group for each LH and VMH cannula placement (n=13 each). Attention was paid to distribute anterior and posterior cannula placements equally into high and low dosage and saline control groups. The number of animals in the LH-control and VMH-control groups was 14. The 15 animals of the sham-operated control group received the footshock and were then handled exactly as the animals given injections.

Twenty-four hours later, in the retest trial, each animal was placed into the large box and the latency to enter the alcove-chamber as well as the total time spent in this chamber during the 5 min period was again recorded.

Step-down avoidance task. The animals were made familiar with the training apparatus in two baseline-trials (one per day), i.e. each animal was put onto the platform which was encircled by a hollow cylinder (30 cm high). The cylinder was kept in place for 10 sec in order to quiet the possibly excited animal. The cylinder was then removed so that the animal could either remain on the platform or step down onto the grid. The training-trial on the third day was identical with the preceding baseline-trials except that upon stepping off the platform the animal received a scrambled footshock of 1 mA for 1 sec. Immediately thereafter the animal was removed from the grid and an injection of SP or saline was administered 30 sec later, according to the injection procedures described above. Both LH-implanted and VMH-implanted animals had been randomly reassigned into experimental and saline control groups: There were again two high dosage SP groups (LH: n=13), VMH: n=11), two low dosage SP groups (LH: n=13, VMH: n=11) and two saline control groups (LH and VMH: n=13 each). Anterior and posterior cannula placements were evenly distributed among all groups.

Twenty-four hours later, the animals were submitted to a retest trial where the latency to descend from the platform was measured. Animals that stayed on the platform for more than 3 min were considered as learners and were therefore removed from the training apparatus.

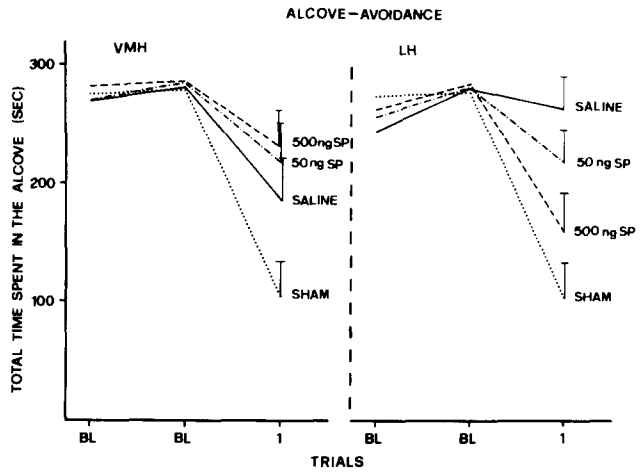

FIG. 1. Effects of posttrial substance P (SP) injection into ventromedial hypothalamus (VMH) and lateral hypothalamus (LH) on learning to avoid alcove after 2 baseline (BL) trials.

RESULTS

All results of statistical analyses are based on comparisons made with the two-tailed Randomization test for two independent samples [22].

Alcove Avoidance Task

The effects of posttrial SP and saline injection into the VMH and LH are shown in Fig. 1. It is clear that either the implantation of the guide cannula or insertion of the injection cannula per se into the LH led to retarded learning since the posttrial LH saline group showed significantly impaired learning compared to sham operated controls ($p<0.001$). This was not the case for the VMH, where the sham-operated controls did not differ significantly from the saline controls.

Whereas in the VMH groups posttrial SP injection showed a (nonsignificant) tendency to impair learning in comparison with saline controls, posttrial SP injection into the LH facilitated learning compared with the saline control group. For the high dosage (500 ng) SP group this difference was significant with a $p<0.03$. I.e. the facilitation of learning with posttrial LH-SP represents a compensation for the learning deficit caused by the cannula implantation.

Step-Down Avoidance Task

Figure 2 illustrates the results of the passive avoidance experiment which was performed following the alcove avoidance task. It is noteworthy that in this task cannula implantation or insertion per se had no influence on learning, since retention of the VMH- and LH-saline controls and the sham-operated controls was about the same. Posttrial injection of SP into the VMH did not significantly influence retention scores, although there was a slight tendency towards enhanced learning. However, posttrial injection of SP into the LH dramatically facilitated learning. Here both doses led to significantly superior learning compared to saline controls, i.e. the high dosage (500 ng) SP group showed enhancement of retention with a $p<0.04$, and the low dosage (50 ng) SP group with a $p<0.001$. Facilitation of learning of the 50 ng SP group was also significant compared to the sham-operated controls ($p<0.01$).

Additionally, analysis of the retention scores of the LH- as well as the VMH-injected animals did not yield any difference with regard to anterior vs posterior injection sites.

After the experiments the brains were sliced and stained and the cannula placements were verified.

DISCUSSION

The results of this investigation extend the finding that SP interacts with learning as previously demonstrated with posttrial SP injection into the substantia nigra [11] and the medial nucleus of the amygdala [10]. The substantia nigra, the amygdala and the hypothalamus belong to those brain regions with particularly high levels of SP [1, 3, 12]. Interestingly, since completion of the present experiments, atlases have appeared showing a more detailed distribution of SP-containing cell bodies and nerve terminals [17]. According to these data, in the LH, including the medial forebrain bundle, SP-positive nerve terminals and fibers of high and medium density were found, whereas the VMH contained cell bodies and only single SP-fibers. It seems reasonable to assume that exogenously applied SP is most likely to influence the activity of those brain areas that contain high densities of SP-positive nerve terminals. The data of the present investigation fit well into this concept, since posttrial injection of SP had a strong facilitating influence on learning in the LH, but failed to yield a clear effect in the VMH. The apparent lack of SP-positive cell fibers or terminals in the VMH could account for these results. Moreover, in both brain regions where a significant influence upon learning was also attained by posttrial SP application, i.e. in the substantia nigra [11] and in the medial amygdaloid nucleus [10], an extremely dense plexus of SP-positive nerve fibers was seen, probably representing nerve terminals [17]

Furthermore, it should be pointed out that in contrast to the amnestic effect of electrical brain stimulation in the substantia nigra [6, 21, 24] and in the amygdala as well [2, 7, 13], posttrial electrical stimulation of the LH led to enhanced memory processing in various passive avoidance tasks [9].

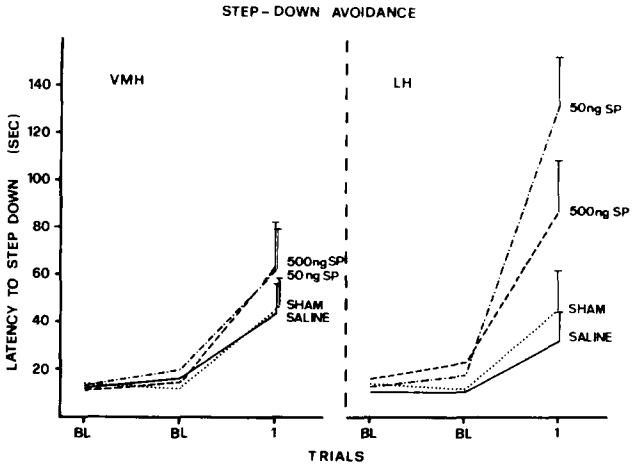

FIG. 2. Effects of posttrial substance P (SP) injection into ventromedial hypothalamus (VMH) and lateral hypothalamus (LH) on learning of a step-down avoidance response after 2 baseline (BL) trials.

The facilitation of learning attained with posttrial SP injection into the LH fits well into this concept.

Taking into account that the effect on learning obtained with posttrial electrical stimulation in the substantia nigra, the amygdala and in the LH parallel the effect of posttrial SP application in these brain regions, it is possible that posttrial substance P imitates the effect of posttrial electrical brain stimulation, as far as memory processing is concerned. This possibility is strengthened by the growing evidence suggesting a transmitter or modulator role of SP [17]. To sum up, SP appears to be one of the most widely distributed putative neurotransmitter peptides, a fact which suggests many sites of interaction between SP and other types of neurons. For example it has been shown that SP, peripherally administered, evokes changes in brain amine turnover [23]. Furthermore there is biochemical evidence for an interaction between SP and catecholamines, since intraventricularly injected SP has been demonstrated to cause the formation of DOPA in several brain regions [16]. In another study an increased release of newly synthetized dopamine in the caudate nucleus following intranigral application of SP has been reported [4]. Recently, in a light-microscopic study, attention has been focused on a possible interaction between catecholamine-containing and SP-containing neurons [18]. The results suggest that SP-containing nerve terminals may contact dendrites or cell soma of catecholaminergic neurons and that SP-containing and catecholaminergic nerve terminals together may influence neuronal activity in some brain regions. Considering the data of the present experiments it is interesting to note that in the VMH only few catecholaminergic and SP-positive fibers could be localized, whereas in the LH high concentrations of SP-positive as well as catecholamine positive fibers were seen [18]. In light of these morphological data and the outlined evidence of a possible interaction between SP and catecholamines, our finding that posttrial SP injection into the VMH has no influence on learning, but SP applied to the LH strongly facilitates learning, can be interpreted in the context of prevailing catecholamine theories of reward and of learning. Considering the large number of putative neurotransmitters in the brain, it is very possible that other or additional interactions between SP and some types other than catecholaminergic neurons may occur which could also, or at least partly, account for the effect of SP on learning.

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