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BRIEF COMMUNICATION

Effect of Substance P on Medial Forebrain Bundle Self-Stimulation in Rats Following Intracerebral Administration[1]

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GOLDSTEIN, J. M. AND J. B. MALICK. *Effect of Substance P on medial forebrain bundle self-stimulation in rats following intracerebral administration.* PHARMAC. BIOCHEM. BEHAV. 7(5) 475–478, 1977. – The effect of Substance P infused intracerebrally via chronically implanted electrode-cannulae on self-stimulation induced from the same site was studied in rats. Substance P caused a significant depression of self-stimulation at 60 and 120 μg/rat. Morphine infused into this site also caused significant depression of self-stimulation, but the doses were considerably lower than those of Substance P (5 and 10 μg/rat). Pretreatment with naloxone, a narcotic antagonist, significantly antagonized the effects of Substance P on self-stimulation. It is proposed that Substance P modulates self-stimulation by the release of an endogenous morphine-like substance, but the possibility of a direct effect of Substance P was not ruled out.

Substance P Morphine Self-stimulation Electrode-cannula

SUBSTANCE P, an undecapeptide, has been shown to have marked effects on the central nervous system. Substance P infusion into the substantia nigra of cats causes an increase of dopamine release in the ipsilateral caudate nucleus [5]; Substance P stimulates monoaminergic neurons in the brain of rats and was proposed as an excitatory transmitter in nerve terminals impinging upon dopaminergic cell bodies [10]; Substance P abolishes the abstinence syndrome in morphinized mice and inhibits aggressive behavior in mice [13]; Substance P produces analgesia after peripheral and central administration in mice [14], and following central administration in rats [11]. The analgesic effects of Substance P were antagonized by prior treatment with naloxone, a narcotic antagonist [11,14].

Substance P is unevenly distributed among regions of the central nervous system and is found in especially high concentrations in the reticular part of the substantia nigra and the interpeduncular nucleus, as well as several septal, preoptic and hypothalamic nuclei [4]; a depolarizing stimulus applied to the hypothalamus was found to release Substance P [7].

Since the foremost central activity of Substance P is depressant and a transmitter function for Substance P has been proposed, the present study was undertaken to determine if another type of behavior, self-stimulation in rats, would be influenced by central administration of Substance P and to compare the effects with those of centrally administered morphine. Self-stimulation was elicited from the medial forebrain bundle at the level of the hypothalamus, and Substance P and morphine were administered intracerebrally into the same site via electrode-cannulae. This electrode site was chosen since the hypothalamus has been shown to contain higher endogenous levels of Substance P compared with other brain regions; the assumption was made that this area would be most sensitive to Substance P application and the electrode-cannula technique would maximize the possibility of observing changes in self-stimulation when Substance P was infused into the same site at which the behavior was elicited.

METHOD

Male Wistar rats, 250–300 g, were anesthetized with Nembutal (50 mg/kg, IP) and stereotaxically implanted with electrode-cannulae (Plastic Products, Roanoke, VA) into the medial forebrain bundle at the level of the lateral hypothalamus (coordinates measured from bregma: posterior 0.6 mm, lateral 1.7 mm, 9.5 mm below the skull). The coordinates used were taken from the stereotaxic atlas of Pellegrino and Cushman [12]. One week following surgery, rats were trained in a standard operant box to press a lever for self-stimulation (stimulus parameters: 60 Hz sine

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wave, 250 msec duration, current range 40–80 μA). Rats were initially trained for daily one-hour sessions until response rates had stabilized, at which time session length was decreased to 30 min and current adjusted to maintain responding between 2,000 and 4,000 responses per 30 min.

The experimental design for intracerebral administration consisted of infusion of sterile water (vehicle) or test drug solution after a 10-min preinfusion period, followed by a 20-min postinfusion period. Infusion was accomplished by insertion of an internal cannula which extended 1 mm beyond the implanted guide cannula (to coincide with the tips of the stimulating electrode, which also protruded 1 mm beyond the guide cannula) and was connected via a length of PE tubing to a Sage infusion pump (infusion parameters: volume 1 μl, rate 0.04 μl/sec, duration 24 sec). The internal cannula was allowed to remain in place an additional 30 sec to permit drug absorption.

In the drug antagonism study, naloxone hydrochloride (20 mg/kg, IP) was administered 10 min prior to intracerebral drug infusion. This dose of naloxone itself did not significantly affect self-stimulation response rates.

Substance P (Peninsula Laboratories, San Carlos, CA) and morphine sulfate were each dissolved in sterile water and infused at various doses, calculated on a microgram per rat basis. At least 3 days for Substance P and 7 days for morphine were allowed between successive doses. Naloxone hydrochloride (Endo Laboratories, Garden City, NY) was dissolved in saline and injected intraperitoneally in a volume of 1 ml/kg. Drug effects were statistically evaluated using a paired Student's *t*-test comparing mean predrug responses to mean postdrug responses at each time period tested (each 30-min session was broken down into two 5-min predrug periods and four 5-min postdrug periods). Data is presented for the postinfusion time period showing the most marked change is self-stimulation response rates (5 min for Substance P, 20 min for morphine).

At the conclusion of the studies, rats were sacrificed with an overdose of Nembutal and electrode-cannulae placement histologically verified. Tips of the stimulating electrodes were found to be within the medial forebrain bundle at the lateral hypothalamic level, dorsolateral to the fornix and medial to the entopeduncular nucleus.

RESULTS

Figure 1 shows the effects of intracerebral infusion of Substance P on self-stimulation. The sterile water vehicle failed to cause any appreciable change in the self-stimulation response rate. Substance P at 3 and 10 μg/rat produced slight but non-significant depression and elevation of self-stimulation rates, respectively. Substance P at 30 μg/rat produced a slight depression, and at 60 and 120 μg/rat produced a significant depression of self-stimulation. Due to the limited solubility of Substance P, the highest dose tested (120 μg/rat) had to be infused in a 2 μl volume; the corresponding vehicle control infusion did not cause any change in self-stimulation.

Morphine also produced depression of self-stimulation following central administration at the same site as Substance P (Fig. 2). Doses of 5 and 10 μg/rat caused significant dose-related depression of responding, whereas 1 μg/rat failed to alter self-stimulation response rates. Once again, the sterile water vehicle was without effect.

The results of the naloxone antagonism study are presented in Fig. 3. Sterile water failed to affect self-stimulation response rates whereas Substance P (60 μg/rat) caused a significant suppression of responding 5 min after intracerebral infusion. Pretreatment with naloxone (20 mg/kg, IP) significantly antagonized the effects of Substance P on self-stimulation. This dose of naloxone alone was without effect on responding for self-stimulation.

DISCUSSION

The results of this study implicate Substance P as a modulator of self-stimulation following central administration. However, a transmitter role for Substance P within the lateral hypothalamus cannot be proposed based on the data obtained from the present study, since (1) Substance P was approximately 12 times less potent than morphine in suppressing self-stimulation, and (2) the minimal effective dose of Substance P that suppressed self-stimulation was 60 μg/rat, a relatively large dose for a proposed neurotransmitter. For comparison, dopamine, a putative neurotransmitter in the nigralstriatal pathway, has an ED50 of 0.6 nanograms/rat for selective inhibition of head-turning

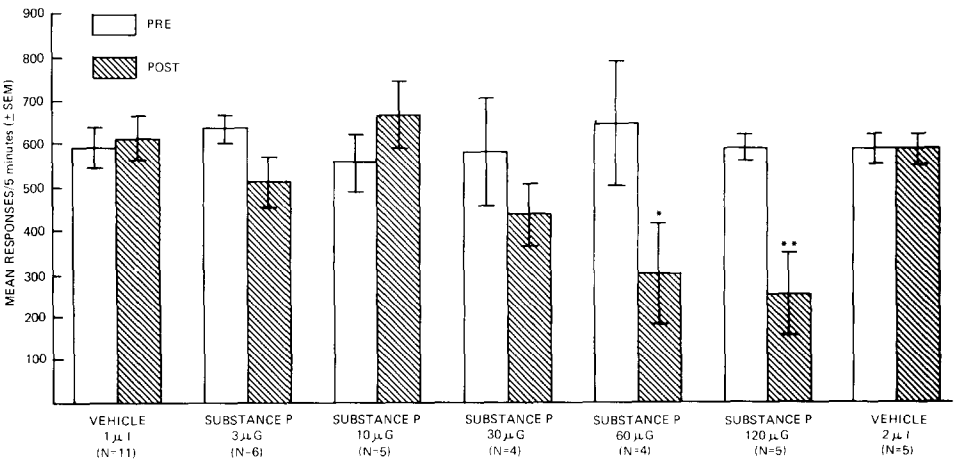

FIG. 1. Effects of Substance P (5 minutes post-infusion) on self-stimulation. *$p<.05$, **$p<.01$ when compared with pre-infusion control value (Student's *t*-test). N: No. of rats.

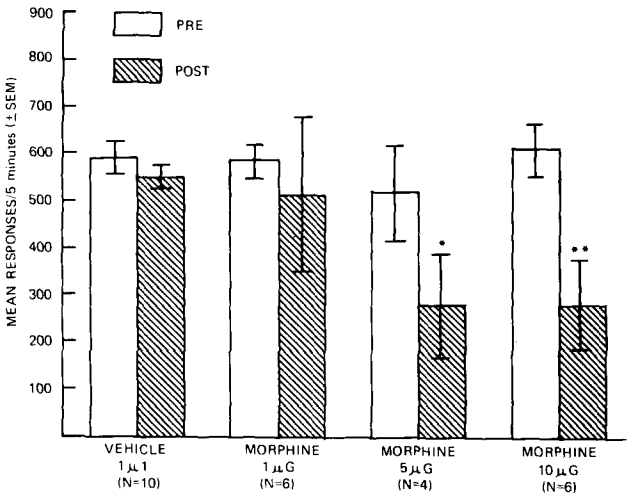

FIG. 2. Effects of Morphine (20 minutes post-infusion) on self-stimulation. *$p<.05$, **$p<.025$ when compared with pre-infusion control value (Student's t-test). N: No. of rats.

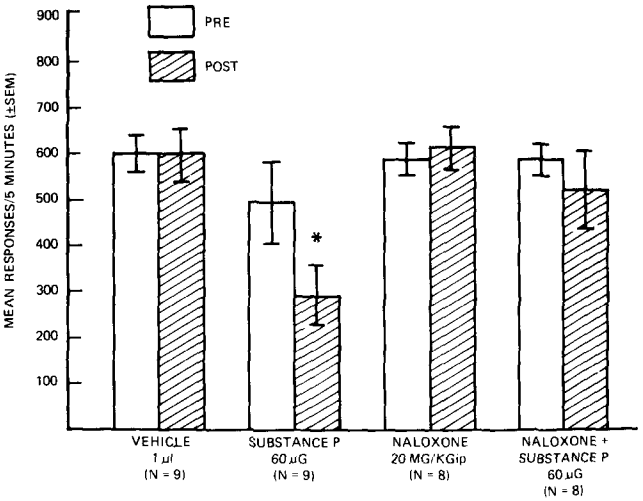

FIG. 3. Naloxone antagonism of Substance P-induced suppression of self-stimulation (5 minutes post-infusion) *$p<.05$ when compared with pre-infusion control value (Student's t-test.) N: No. of rats.

induced by electrical stimulation of the caudate nucleus [1]; in this study, dopamine was also administered via an electrode-cannula.

On the other hand, there is evidence which would imply that Substance P is releasing another endogenous morphine-like substance, and this substance in turn could be causing the observed depression of self-stimulation: (1) Substance P causes analgesia at the same site as morphine (central gray) [11], but Substance P does not bind to the opiate receptor [15]; (2) Naloxone antagonizes morphine- and Substance P-induced analgesia [11], indicating that a common receptor may be involved (directly with morphine, indirectly with Substance P); and (3) Naloxone antagonizes Substance P-induced suppression of self-stimulation. Two possible candidates for this endogenous substance are methionine- and leucine-enkephalin, since Belluzzi and Stein [2] have shown that the enkephalins can depress self-stimulation when infused intraventricularly; this possibility appears highly unlikely since relatively high doses of enkephalins were necessary to suppress self-stimulation and the analgesic duration of activity of Substance P was found to be much longer than for the enkephalins [11]. Another endogenous candidate is β-endorphin. β-Endorphin (C-fragment of β-lipotropin; β-LPH 61–91) has been shown to produce potent, long-lasting analgesia following intracerebral administration in rats [3, 6, 9]. Although there is no direct evidence that Substance P can induce the release of endorphins, studies are in progress to determine if β-endorphin can suppress self-stimulation when infused into the same site.

Finally, there is evidence that Substance P interacts with monoaminergic systems. Substance P has been shown to release dopamine in the caudate nucleus following its administration into the substantia nigra [5] and to cause the formation of Dopa in several brain regions [10]. However, if dopamine was the released substance, then self-stimulation rates should have been increased following Substance P rather than decreased since both L-Dopa and apomorphine caused increases in self-stimulation rates from medial forebrain bundle electrodes [8].

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