5-7-1991

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BILATERAL INJECTIONS OF SUBSTANCE K INTO THE MEDIAL PREOPTIC AREA HAVE NO EFFECT ON MALE RAT SEXUAL BEHAVIOR

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May 7, 1991

INTRODUCTION

Within the last five years, there has been increasing interest in the role of neuropeptides in the neuroregulation/modulation of both male and female sexual behavior (2). The impetus for this derives from several findings. Firstly, certain neuropeptides and their receptors are located in brain loci previously implicated in both male and female sexual behavior (14). Secondly, and most important, is the rapidly accumulating evidence that the concentrations of certain peptides within these behaviorally-relevant loci are regulated by gonadal steroids (10,15).

One peptide of particular interest in regard to male rat sexual behavior is substance P (SP). Substance P is found in a richly interconnected circuit which includes the bed nucleus of the stria terminalis (BnST), medial amygdala (mAMY), and medial preoptic area (MPOA). These cell groups have been shown to play an integral part in the expression of male copulatory behavior (13). Moreover the concentrations of SP within these nuclei have been shown to be dependent on circulating levels of steroids (6). For example, castration produces a significant reduction in SP immunoreactivity in the medial amygdala in male rats (8), and hamsters (6). Most importantly, numerous studies have demonstrated the critical importance of the MPOA for male sexual behavior in a variety of mammalian

species, including primates (13) and it has now been demonstrated that castration reduces SP immunoreactivity in this region as well. Accumulating evidence also indicates that the concentrations of sP within this interconnected circuit is sexually differentiated (10,14). For example, Micevych et al. (10) reported that in rats, SP concentrations in the posterior segment of the amygdala were higher in males than in females. Additionally, Malsbury and McKay (8) recently reported a sex difference in the pattern of SP staining in the medial segment of the BnST. Collectively, this suggests that perhaps sP play a part in the neural modulation/regulation of male copulatory behavior. Indeed, in a study by Dornan and Malsbury (4) they reported that bilateral injections of 10, 100, 1000 ng of sP directly into the MPOA-AH reduced the latencies to initiate copulation. Injections of the two lower doses also reduced ejaculation latencies. Bilateral injections of sP (10 ng/cannula) into the dorsal caudate putamen, however, had no appreciable effect on male copulatory behavior, while injections into the lateral ventricles produced changes different from those of MPOA-AH injections. These results argue for some degree of site specificity of the effects of MPOA-AH injections of SP. The facilitatory effects of SP on male copulatory behavior seemed to be dependent on circulating levels of androgens as MPOA-AH injections of 10 ng of SP in

castrated testosterone-deprived animals failed to reverse the decline in male copulatory behavior that is normally observed following castration.

Substance K, a novel member of the tachykinin family of peptides, has been shown to be structurally similar to substance P. Furthermore, reports have shown that in some areas of the brain, substance K and substance P are co-synthesized and/or co-released. The discovery of SK has spawned much interest and research. One important result was the discovery that both SP and SK are produced from the same prohormone, beta-preprotachykinin (7). The chemical structure of the two peptides is thus very similar and their sequences differ by only a few segments (7). These results suggest that both tachykinins could be co-synthesized and/or co-released and there has been strong support for this possibility (7).

The results of binding studies have revealed that SK and SP are widely distributed in the brain but that their local densities differ. For example, SK has many more binding sites in the ventral mesencephalon than SP (7,9). Both peptides display their highest densities in the substantia nigra (7,9) but there are few actual binding sites in this area for SP, while there are many for SK (7).

In contrast to the reported behavioral effects of sP, the behavioral effects of SK are rather unknown and reports are limited. When infused into dopaminergic areas

such as the ventral tegmental area or the stria terminalis, SK has been shown to cause an increase in locomotor activity greater than that seen with SP (1). Burns and Kelley (1) administered SK into the ventral tegmental area of rats and observed an increase in locomotor activity in an open field environment. This effect was subsequently blocked with haloperidol, a dopamine receptor blocker, confirming the facilitory effect of SK on dopaminergic neurons. A recent report on operant responding revealed that both SK and SP stimulated general motor activity but had no effect on patterns of lever pressing (5).

Presently, we are unaware of any reports in the literature of the role of SK on sexual behavior. This is most likely due to its relative infancy of discovery. Indeed, the compositions of SK and SP are so similar that the technology is still emerging to distinguish between their receptors. This has important implications since it is quite possible that some previous behavioral effects attributed to SP may actually be more accurately associated with SK or even another novel tachykinin, neurokinin B.

It is appropriate then to try to ascertain the behavioral effects of SK by testing it in areas of the brain previously shown to be responsive to SP. As previously mentioned, one such area of SP influence is the medial preoptic area, as reported

by Dornan and Malsbury (2). At present, however, it is not known whether Substance K hat the level of the medial preoptic nucleus as any role in the neural regulation of male rat copulatory behavior. Thus, it is appropriate to test the effects of substance K in a specific brain area where substance P has been previously implicated in the neural control of male rat sexual behavior. The medial preoptic area (MPOA) was chosen because substance P has been shown to modify sexual behavior in the male rat when inducted in this area.

METHODS

Animal and Surgery

Eighteen adult male Long-Evans rats were obtained from the laboratory colony (Illinois Wesleyan University, Psychology Department, Bloomington, IL.). Animals weighed between 360 and 440 grams at the time of surgery. The animals were singly housed in a controlled environment. Light/dark cycle was maintained with lights on at 6:30 and off at 21:30. Each subject was regularly held so as to minimize stress during injections and other procedures. Food and water were provided ad lib.

Ovariectomized females of the same strain were used as male stimuli. Forty-eight hours prior to testing each female was subcutaneously (sc) injected with 10 micrograms (ug) of estradiol benzoate and then received 1 ug of progesterone (sc) four to five hours before testing. This procedure was done to ensure maximal sexual receptivity (2).

Each male was given preoperative sexual experience by placing it with a sexually receptive female. Each male was allowed two preoperative sessions and all animals had at least one ejaculation. No exclusions for continuing the

experiment were made based on levels of sexual response.

Five to ten days after the second preoperative session, each male was anesthetized with pentobarbital sodium (Nembutal, 40 mg/kg) and received a pair of stereotaxically implanted 22-gauge stainless steel guide cannulae (13 mm long) with inner stylets to prevent occlusion. The cannulae were aimed 2 mm above the medial preoptic area (from Bregma, AP = +1.8; ML = + 0.6; DV = -5.9) using the atlas of Paxinos and Watson (12) as a guide but with the incisor bar adjusted to +5.0. The guide cannula were secured to the skull with dental acrylic cement and stainless steel screws.

Behavior Testing

Following surgery, rats were allowed five to eight days recovery before behavioral testing resumed. During testing, males were placed in a rectangular test arena (20 x 15 x 14 inches) with corn cob bedding following intracerebral injections. The subject was given five minutes to adapt to the box before a steroid-primed receptive female rat was placed in the cage. Females were verified to be receptive before the session by the presence of a lordosis response when mounted by a non-experimental male from the lab.

Several parameters of male copulatory behavior were measured during testing including: mount latency (ML), the interval from introduction of the female to the first mount by the male; intromission latency (IL), the interval from the introduction of the female to the first intromission; ejaculation latency (EL), the interval from the first intromission and the first ejaculation; the post-ejaculatory interval (PEI), the interval from ejaculation to the first mount by the male; mount frequency (MF), the number of mounts without intromission between the introduction of the female and ejaculation; and intromission frequency (IF), the number of intromissions between the introduction of the female and ejaculation.

Animals were allowed 30 minutes to achieve an intromission following introduction of the female. Those that failed to do so were assigned latencies of 1800, the duration of the observation period to that point. If an animal had an intromission within 30 minutes, it was allowed a total of 60 minutes to achieve ejaculation. The test concluded following the first mount after ejaculation (PEI).

Each rat was assigned three post-operative tests, scheduled one week apart. Prior to testing, each animal received either saline or one of two doses of substance K. Animals received all three of the doses over three tests in random order. During testing, behavioral parameters were recorded on an IBM-compatible

personal computer. Half of the sessions were videotaped and reviewed to ensure accurate behavioral recording. Due to limitations of the equipment, all of the sessions could not be videotaped.

Intracerebral Injections

Rats were removed from their cages individually and moved to a low noise and light environment, where stylets were removed. Injections were made with a 26-gauge inner cannulae (15 mm long, extending 2 mm past the guide) which was attached to a 2 microliter Hamilton syringe by a plastic tube. Before the injection, saline solution was drawn through the tube until 1 ug of liquid could be drawn and expelled. An air bubble was then drawn through the injector so that the peptide or saline solution could be separated from the saline when it was subsequently drawn. Half a microliter of either 10 or 1000 ng of substance K (Sigma Chemical Co., St. Louis, MO.) in a 9% saline solution alone was manually injected over 60 seconds into each side of the brain. During injections, movement of the air bubble ensured that the peptide or saline solution had actually been injected. If a discrepancy arose as to the movement of the air bubble, another dose was administered. The inner cannula injector was left in place after completing the injection so that the solution

would diffuse into the tissue rather than upward. After the procedure was complete on both sides, the stylets were replaced and the animal was placed in the test box for five minutes until testing began. All peptide and saline solutions were prepared immediately before each test session.

Histological Analysis

At the conclusion of data collection, all animals were given an overdose of Nembutal and sacrificed. Brains were removed and placed in a cryostat where 40 micron sections were obtained to analyze cannula track locations using a cresyl violet stain.

RESULTS

Of the 20 animals that began the study, one was never tested due to illness, one animal was tested only once due to cannula blockage, and five did not receive the third test due to cannula problems or sickness. Of the 13 remaining animals, three did not receive all three solutions and one did not have both cannulae placements within the MPOA (see figure 1 for schematic representation of placement sites), leaving 9 animals for data analysis.

A one way ANOVA measuring latencies by dose revealed that substance K failed to produce a significant effect on mount latency ($F = 0.88$, $p > 0.05$), shown in figure 3; intromission latency ($F = 0.96$, $p > 0.05$), shown in figure 2; and ejaculation latency ($F = -0.01$, $p > 0.05$), figure 4.

DISCUSSION

The results of this study reveal that bilateral injections of substance K into the MPN had no apparent effect on any parameter of male rat copulatory behavior. However, the animals in each test that received the 10 ng dose of peptide appear to be different from both of the other groups when a visual inspection of he figures is made. It is only speculative, but perhaps the 1000 ng dose is too high to have an effect. It would be beneficial to test this possibility by selecting a dose between 10 and 1000 ng as well as one less that 10 ng in a future experiment.

One criticism a reader may have is the sexual inexperience of the sample and the lack of homogeneity that a selection procedure may offer. It is true that screening procedures would control the variance in the population that was used but this variance in sexual experience of males was desired in this study. The reason for this was the novelty of substance K testing on sexual behavior. It was unknown beforehand whether our manipulations might have a facilitory or inhibitory effect. The measured effect of substance K would be easier to detect in a sample of varying degrees or sexual vigorousness. Until some indication of the peptide's effect is discovered, it is not appropriate to do more elaborately controlled research.

Thus, this study served as a pilot project.

These results, however, are consistent with a study by Kafra (in press), which reports that intracerebral ventricular injections of substance K has no effect on male sexual behavior. The implications of these results, coupled with this project are that substance P has a specific role in controlling male rat sexual behavior, while substance K does not. Of course, to verify this possibility requires much more research. The results of the present study have some limitations so the role of substance K in the MPOA needs clarification, as well as its role in other important areas controlling sexual activity, including the bed nucleus of the stria terminalis and the medial amygdala. With so little known about the behavioral effects of substance K, there is certainly much work to be done.

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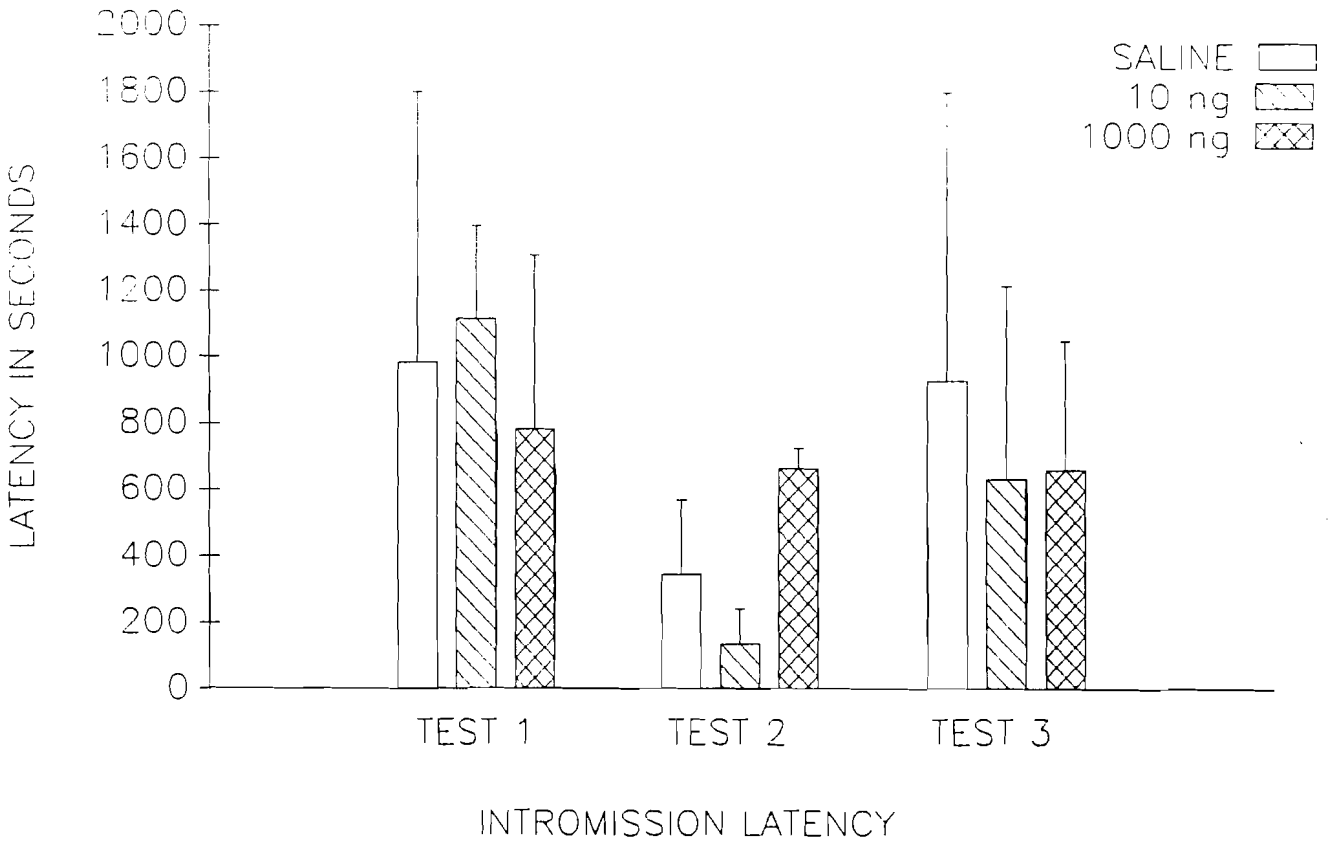
Figure 1. Schematic representation of the medial preoptic nucleus. All bilateral cannula placements in males which received injections of substance K or saline were aimed at this area. For illustrative purposes only (the boundary of the MPN) a schematic from Matuszewich and Dornan (Psychopharmacology, In Press) was used from which the figure is redrawn. Abbreviations: ac, anterior commissure; cc, corpus callosum; CPu, caudate putamen; GP, globus pallidus; f, fornix; ic, internal capsule; 3V, third ventricle; MPN, medial preoptic nucleus; oc, optic chiasm.

Figure 2. Effect of bilateral injections of 10 ng or 1000 nf (n=9) of substance K or saline (n=9) into the medial preoptic nucleus on intromission latencies. See text for full details. Vertical lines indicate $\pm$ standard error of the mean.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LATENCY IN SECONDS
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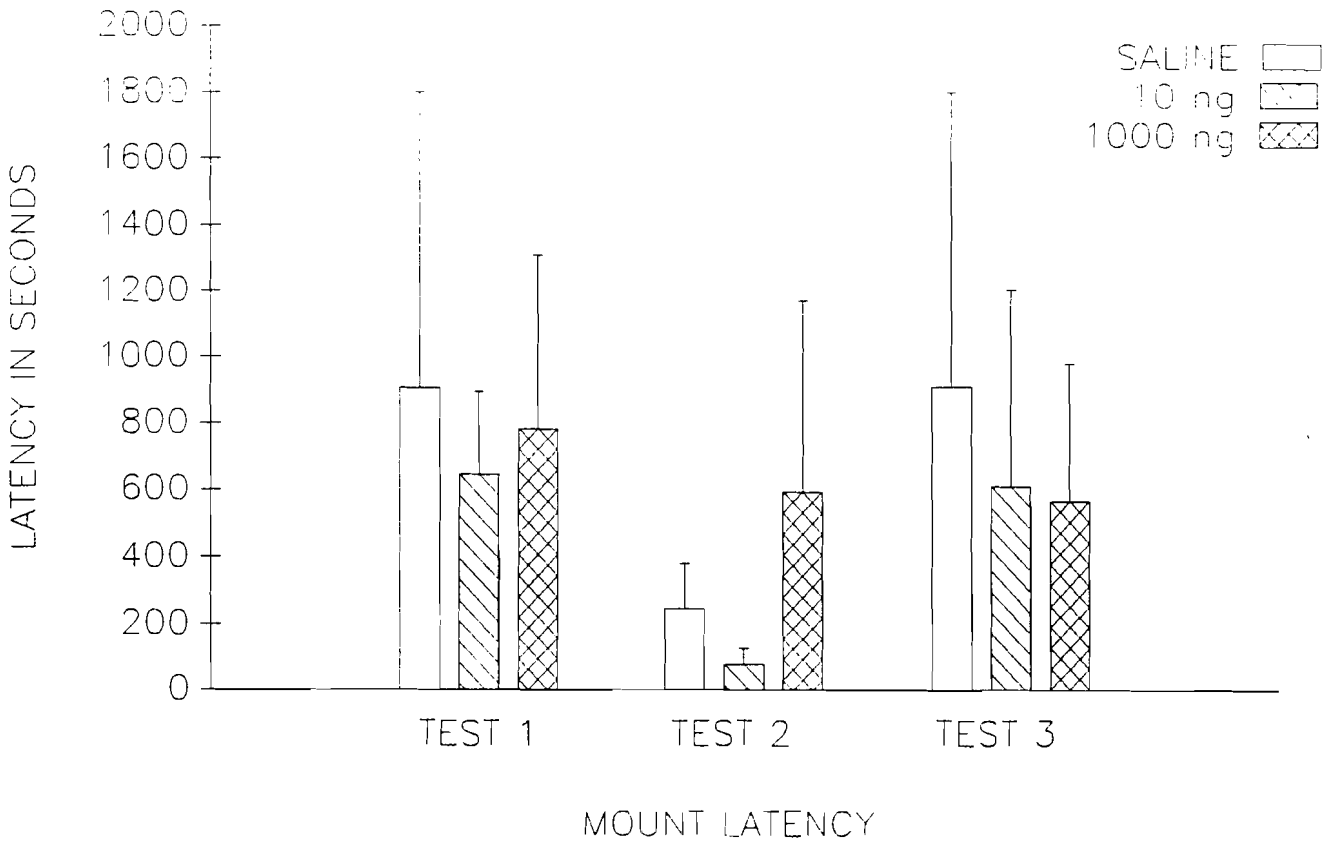
TEST 2
TEST 3
INTROMISSION LATENCY

Figure 3. Effect of bilateral injections of 10 ng or 1000 nf (n=9) of substance K or saline (n=9) into the medial preoptic nucleus on mount latencies. See text for full details. Vertical lines indicate $\pm$ standard error of the mean.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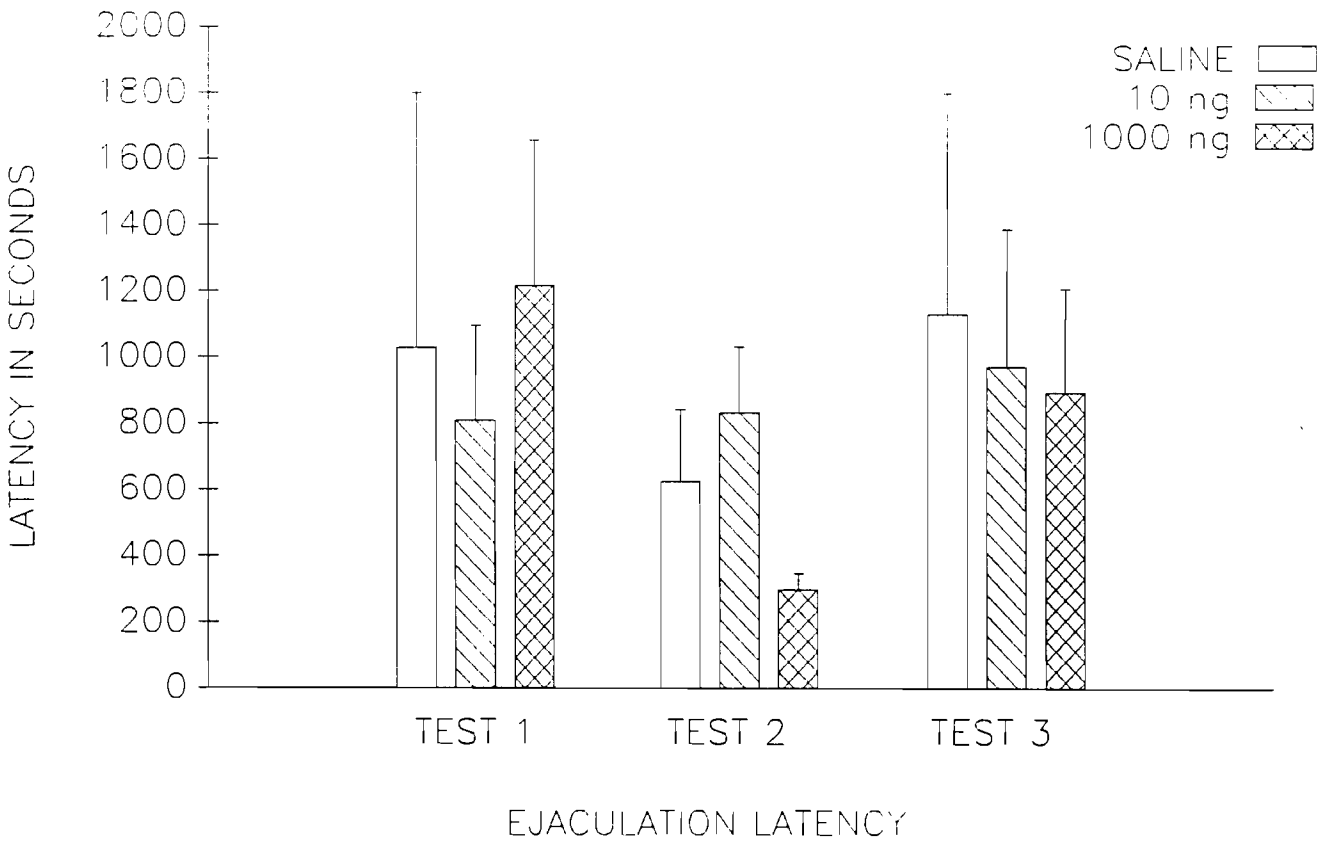
LATENCY IN SECONDS
SALINE
10 ng
1000 ng
TEST 1
TEST 2
TEST 3
MOUNT LATENCY

Figure 4. Effect of bilateral injections of 10 or 1000 ng of substance K 500 (n=6), or or saline into the medial preoptic nucleus on the ejaculation latency. See text for full details. Vertical lines indicate $\pm$ standard error of the mean.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