

## CENTRAL INJECTION OF SUBSTANCE P ELICITS GROOMING BEHAVIOR AND MOTOR INHIBITION IN MICE

R.J. KATZ

*Mental Health Research Institute, Department of Psychiatry, University of Michigan Medical Center, Ann Arbor, MI 48109 (U.S.A.)*

(Received October 20th, 1978)

(Revised version received December 19th, 1978)

(Accepted December 19th, 1978)

---

### SUMMARY

Substance P undecapeptide (SP) was injected into the lateral ventricles of adult male Swiss-Webster mice. Two behaviors were observed after peptide. SP increased grooming in a naloxone non-reversible manner and reduced exploratory motor activity. Both effects were dose dependent. Both effects may point to psychoactive properties for SP.

---

Recent interest in substance P (SP) as a putative central nervous system (CNS) neurotransmitter has prompted a variety of physiological and neurochemical experiments [11]. Only a limited behavioral assessment of this peptide has been carried out, however, although some effects upon nociception, aggression and some stereotyped motor behavior have been reported [4,5,7,8,10]. This report summarizes our observations on central injection of SP-induced grooming and motor activity. We examined grooming and motor activity based upon past reports with other peptides (e.g. ref. 3).

*Subjects.* Adult male Swiss-Webster mice 25–40 g each were maintained upon ad libitum food (Teklad 4.0% fat rodent diet S-0836) and tap water, and automatically programmed day/night cycles of 12 h (lights on = 8:00–20:00 h). A minimum of 5 mice each were used to determine a given data point.

*Injection.* SP (Arg-Pro-Lys-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH<sub>2</sub>, Peninsula 7451, San Carlos, CA) 0–100 µg, was infused in Ringer-Locke solution. The injection was into the lateral ventricle through a permanently indwelling 23G cannula which was implanted stereotaxically under 80 mg/kg sodium pentobarbital (Nembutal) a minimum of 7 days preceding injection. Additional details of cannula construction and implantation have been published previously [6]. Injections utilized a manually operated Hamilton microsyringe and an infusion volume of 10 µl over a period of 30 sec or less. Doses were based

upon previous reports [10]. To further examine the pharmacology of SP-induced grooming a dose known to elicit this behavior was also tested after pretreatment with 8 mg/kg of naloxone HCl, injected intraperitoneally 1 ml/0.1 kg 10 min prior to SP.

*Procedure for behavioral observations.* Observations of grooming and motor inhibition were made at the same time. Subjects were injected and placed in 51 × 41 × 22 cm polypropylene cages (Scientific Products Series 70) which contained a fresh bedding of pine chips. The cages rested upon 4 field sensitive activity monitors (Stoelting, Chicago, IL) calibrated to within 5% of each other. Additional details of apparatus and procedures have been previously published [6]. Observations of grooming were rated for the first 20 min after injection on a 0–3 scale with 0 = no observed grooming, 1 = 300 sec or less of observed grooming, 2 = 300–600 sec of observed grooming, 3 = more than 600 sec of observed grooming over a 20-min observation period. Grooming was scored for face washing, flank washing, and use of hindpaws for flank or head scratching. Neither yawning nor stretching was prominent in control or drug injected subjects and therefore they were not scored. Motor activity was recorded automatically for 12 consecutive 10-min intervals using the apparatus described.

*Results.* SP facilitated grooming (Fig. 1) and decreased other motor activity (Fig. 2). In both cases the drug effect was dose dependent. Randomized design analyses of variance [1] and two factor mixed design analyses of variance [1] indicated both effects, respectively, were statistically significant. F ratio for the drug effect upon grooming was  $F_{4,30} = 7.92$ ;  $P < 0.001$ . Pretreatment of mice with naloxone had virtually no effect upon the grooming response at a dose of 50  $\mu$ g SP (mean scores with and without naloxone = 3 in both cases\*). For motor activity (Fig. 2) ratios for drug effects upon time and dose were  $F_{4,30} = 3.1$ ,  $F_{12,361} = 12.94$  respectively,  $P < 0.001$ , in each case. The interaction effect was not significant;  $F_{48,361} = 1.0$ ,  $P < 0.50$ . While main effects upon motor activity were significant and represent a general decline in activity, it should be noted that a minority of SP-injected mice showed behavioral activation especially at higher ( $>25 \mu$ g) doses. Approx. 1/10 of the subjects tested showed augmented motor activity and a Straub tail response (i.e. tail elevation) indistinguishable from typical murine running to opiates [5]. Head to tail turning was also observed in some animals.

Several reports have suggested that SP may have sedative or behaviorally depressant properties [6,10]. We have noted a decrease in motor activity in

---

\*Further evidence for the independence of SP grooming and opiate blockade was recently found in our laboratory. In a 10-min test using 50  $\mu$ g of SP a saline pretreated group showed  $266 \pm 52$  sec of grooming and a group pretreated with 1 mg/kg of Naltrexone showed  $162 \pm 39$  sec, (all values as mean and standard error) (using Student's *t*-test,  $t = 0.8$ ,  $P > 0.20$ ,  $n = 10$ ,  $n = \text{subj./group}$ ). This slight and non-significant reduction is considerably smaller than Naltrexone reductions in grooming after ACTH fragments which have previously been seen by other observers (e.g. ref. 3). I am indebted to Kevin Roth for these data.

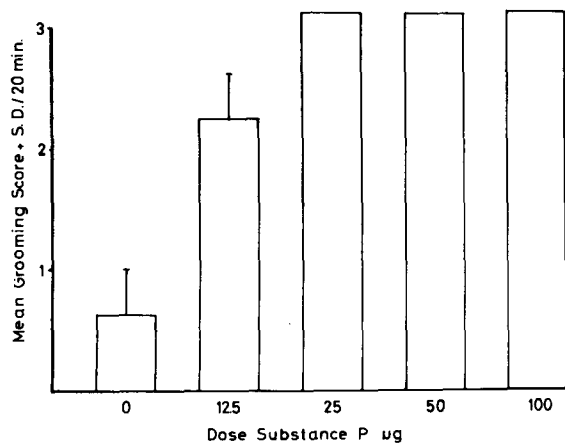


Fig. 1. Induction of grooming in mice by central SP-injection as judged by visual observation. A 0–3 scale was used, with 3 a maximal score (i.e. >50% of observation period) additional details in text.  $n = 7/\text{group}$ .

the bulk of mice we have tested. The present results qualify previous global assessments because not all motor behaviors are reduced. For example, grooming was increased.

Previous reports have also noted increases in occurrence of certain motor behavior which may agree with the present findings. The non-directed licking in cats seen after SP may be another aspect designated as grooming here [10]. The present observations are consistent with another recent report [7] observing a number of similar behavioral effects across a course of injections

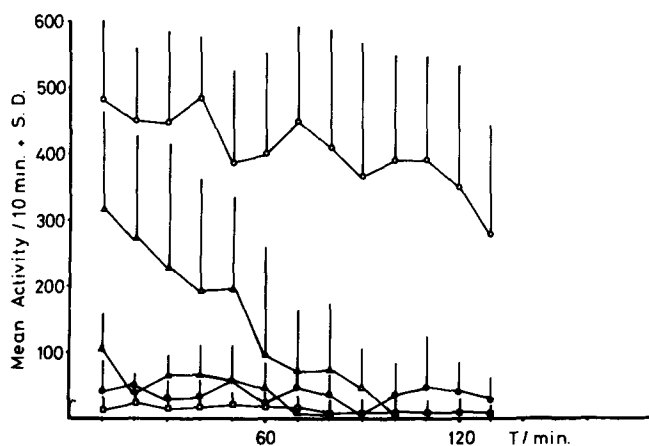


Fig. 2. Reduction of motor activity in mice by SP which was recorded automatically using a commercially available system of tuned oscillators (Stoelting; see ref.6). Subjects were placed in apparatus at time,  $T = 10$ . 10-min intervals were used throughout. o, Ringer Locke (RL) solution;  $\Delta$ , substance P 12.5  $\mu\text{g}$ ;  $\blacktriangle$ , substance P 25.0  $\mu\text{g}$ ;  $\bullet$ , substance P 50  $\mu\text{g}$ ;  $\square$ , substance P 100  $\mu\text{g}$ .  $n = 7/\text{group}$ .

into the substantia nigra. Both species and procedural differences may account for different time courses. Both present and past findings may be interpreted in terms of recent electrophysiological evidence showing SP and enkephalins exerted similar actions upon a common receptor [2]. ACTH fragments which also elicit grooming have a high affinity for opiate receptors [3]. Nonetheless, the failure to observe naloxone reversal suggests that SP did not compete for opiate receptors in the present investigation.

#### ACKNOWLEDGEMENTS

Dr. Katz was supported by grant MH 07417 from the National Institute of Mental Health through the Mental Health Research Institute. The technical assistance of Guilo Baldrighi and editorial assistance of Esther Washington are gratefully acknowledged.

#### REFERENCES

- 1 Bruning, J.L. and Kintz, B.L., *Computational Handbook of Statistics*, 2nd edn., Scott Foresman and Co., Glenview, Illinois, 1977.
- 2 Davies, J. and Dray, A., Substance P and opiate receptors, *Nature (Lond.)*, 268 (1977) 351-352.
- 3 Gispen, W.H., Reith, M.E.A., Schotman, P., Wiegant, V.W., Zwiers, H. and de Weid, D., CNS and ACTH-like peptides: Neurochemical Response and Interactions with Opiates. In C.H. Miller, C.A. Sandman and A.J. Kastin (Eds.), *Neuropeptide Influences on the Brain Behavior*, Raven Press, New York, 1977, pp. 61-80.
- 4 Henry, J.L., Substance P and pain: a possible relation in different transmission. In U.S. von Euler and B. Pernow (Eds.), *Substance P*, Raven Press, New York, 1977.
- 5 James, T.A. and Starr, M.S., Behavioral and biochemical effects of Substance P injected into the substantia nigra of the rat, *J. Pharm. Pharmacol.*, 29 (1977) 181-182.
- 6 Katz, R.J., Carroll, B.J. and Baldrighi, G., Behavioral activation by enkephalin in mice, *Pharmacol. Biochem. Behav.*, 8 (1978) 493-496.
- 7 Kelley, A.E. and Iversen, S.D., Behavioral effects of substance P injections into long reticulata of substantia nigra, *Neurosci. Lett., Suppl.*, 1 (1978) 220.
- 8 Krivoy, W., Kroeger, D. and Zimmermann, E., Analgesic actions of substance P. In U.S. von Euler and B. Pernow (Eds.), *Substance P*, Raven Press, New York, 1977, pp. 195-200.
- 9 Olpe, H.R. and Koella, W.P., Rotatory behavior in rats in intranigral application of substance P and eledoisin fragment, *Brain Res.*, 126 (1977) 576-579.
- 10 Von Euler, U.S. and Pernow, B. Neurotropic effects of substance P, *Acta physiol. Scand.*, 36 (1954) 265-275.
- 11 Von Euler, U.S. and Pernow, B., Substance P, *Nobel Symposium 37*, Raven Press, New York, 1977.