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ENKEPHALIN-DOPAMINE INTERACTIONS IN BEHAVIORAL AROUSAL

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Abstract

1. Central injection of a long lasting enkephalin analog (D-Ala^2-leu-enkephalin-amide) produced a syndrome of stereotyped motor activity.
2. This activation syndrome was significantly reduced by two dopamine (DA) blockers (pimozide, fluspirilene) and by an agonist of inhibition-mediating DA receptors [(3,4-dihydroxyphenylamino)-2-imidazoline].
3. Intact DA functioning is necessary for the occurrence of this enkephalin-mediated behavioral syndrome.

Key words: dopamine, dihydroxyphenylamino-imidazoline, enkephalin, fluspirilene, pimozide, psychomotor activity

Abbreviations: DA DPI ICV

Introduction

Enkephalins and long lasting synthetic analogues are known to produce a syndrome of behavioral activation in mice. This syndrome consists of continual stereotyped running, an absence of vertical rearing, Straub tail, and a loss of environmental orientation (e.g., mice will repeatedly collide with cage walls or other obstructions in their path) (Katz, et al., 1978; Wei, et al., 1977). The neurotransmitter DA has been shown to control many forms of motor activation (Barbeau, 1973; Papeschi, 1972), and recent studies suggest both dense binding of opiates within DA rich areas of the central nervous system, and physiological activity for enkephalins in association with dopaminergic cell bodies and terminal areas (Pollard, et al., 1977; Dafny and Burks, 1976; Dill and Costa, 1977). Since dopamine-containing neurons directly affect activity, and since DA occurs in conjunction with enkephalin containing neurons which also produce activation, we investigated whether enkephalin induced behavioral activation was dependent upon intact DA functioning.

Our investigation consisted of pretreating mice with specific DA receptor blockers: (pimozide, fluspirilene) (Anden, et al., 1970) and an agonist of inhibitory DA receptors, DPI [(3, 4-dihydroxyphenylamino)-2- imidazoline] (Cools, et al., 1976) and injecting a standard dose of enkephalin analogue known to produce running behavior (Katz et al., 1978). Comparison was made with vehicle pretreated enkephalin injected mice.

Methods

Animals. Eighteen adult male Swiss-Webster outbred mice (30-40 g each) were obtained locally (Charles River, Portage, MI) and maintained in a standard laboratory environment with food and water available ad libitum and normal lighting cycles of 12 h light/12 h darkness.

Drugs. Enkephalin analogues were injected intracerebroventricularly (ICV) at a fixed time after drug treatment, and this time varied with the nature of pretreatment. DPI, the DA blockers and the vehicle solution were injected intraperitoneally 10 ml/kg. Pimozide in doses from 0.00-3.00 mg/kg was injected in a 0.3% tartaric acid vehicle 20 min prior to enkephalin injection; fluspirilene in doses ranging from 0.00-5.00 mg/kg was injected suspended in a 0.9% saline vehicle 40 min prior to central injections; DPI 0.00-10.00 mg/kg was injected in a 0.9% saline vehicle 20 min prior to ICV injection. A standard dose for 25 µg of d-Ala^2-leu-enkephalin-amide (Peninsula Laboratories, San Carlos, CA) was injected ICV in 10 µl of Ringer-Locke solution. Central injections were performed manually using a Hamilton micro-syringe and an infusion time of less than 30 sec.

Procedures. Subjects were anesthetized with 80 mg/kg of sodium pentobarbital (Nembutal) and stereotaxically implanted with a cannula aimed at the third ventricle of the brain. The cannula consisted of a stainless steel 23 g needle and was mounted to the skull with three stainless steel screws and acrylic dental cement. During the week of recovery from surgery, subjects were placed in the experimental-recording apparatus for five 2 h habituation sessions.

The experimental apparatus consisted of 4 field sensitive platforms (Stoelting, Chicago) calibrated to within 5% of each other. Mice were individually placed in polypropylene cages (Scientific Products series 70; 51 x 41 x 22 cm) which rested directly upon the platforms. Fresh pine chip bedding was placed in each cage. Test sessions consisted of an initial 1 h habituation at the close of which drugs or vehicle solution were injected intraperitoneally.

A within-subjects design was used to evaluate drug effects, i.e., subjects (6/drug evaluation) were tested with 2 control injections (vehicle and Ringer-Locke injection, vehicle and enkephalin analogue injection) and also with 3 doses of drug and enkephalin. The various treatments were administered in a counterbalanced order. Drug injections were separated from each other by at least 7 days. We have noted only minor (10%) behavioral tolerance using this procedure. Ten minute recording intervals were used throughout, and comparison was based upon percentage transformed scores.

Statistical analysis: due to initial heterogeneity of variance across cells statistical analysis way by a non-parametric procedure. The Friedman two-way analysis of variance technique for related samples, using a criterion of $p < .05$ was used as the basis of statistical comparison.

Results

All results were analyzed via Friedman Analysis of Variance (Siegel, 1956). Results are summarized in Fig. 1 A-D. Fig. 1A presents an untransformed summary of all vehicle pretreated leu-enkephalin analogue injections compared with Ringer-Locke injections. There is a significant elevation of activity persisting for at least 60 min. $\chi^2_r = 6$; $p < .05$, $df = 2$. Figure 1, sections B, C, and D present the effects of pimozide, fluspirilene, and DPI respectively upon the 1 h time period following central enkephalin injection. It is clear in all 3 cases that a monotonic reduction in behavioral activation follows increasing DA receptor inhibition. All drug effects were significant by analysis of variance ($\chi^2_r = 10.6, 14.3, 14.1$, $p < .02, .01, .01$ respectively, $df = 3$). Direct visual observations of enkephalin treated animals under all drug conditions indicated that Straub tail elevation was unaffected by any DA blocker. Although these mice were quiescent, they occasionally (upon a loud noise) showed a brief explosive burst of forward locomotion. In all other respects, drug pretreated animals were similar to vehicle pretreated animals.

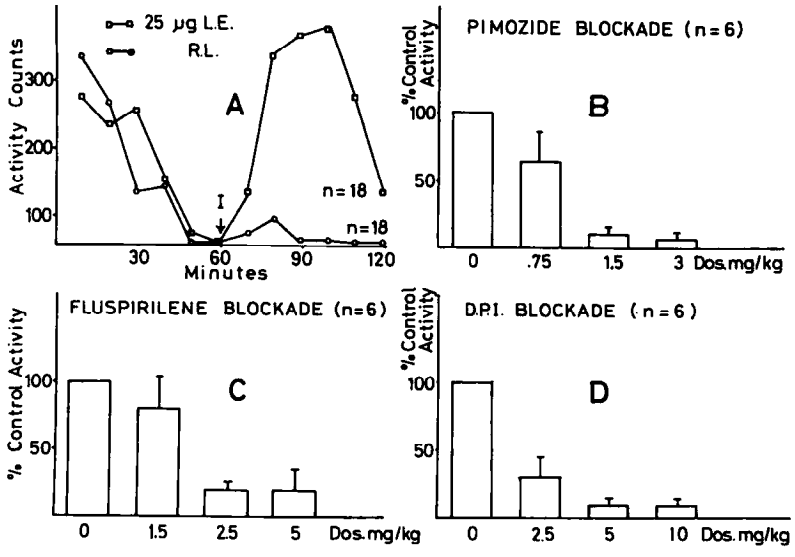

Fig. 1. Enkephalin-dopamine interaction. Effects of DA manipulations upon activity induced by a long lasting enkephalin analogue.
A: Enkephalin induced behavioral activation (all scores as means). I denotes intraventricular injection; LE: Leu-enkephalin analogue RL: Ringer-Locke solution.
B: Effects of pimozide upon enkephalin activation.
C: Effects of fluspirilene upon enkephalin activation.
D: Effects of (3,4-dihydroxyphenylamino)-2-imidazoline upon enkephalin activation. Scores for B, C and D as % mean ± % standard error.

Discussion

Our results suggest that the behavioral effects of enkephalins are dependent at least in part upon intact DA systems for their expression. Earlier findings from our laboratory have shown similar interactions for morphine and dopamine in murine running (Carroll and Sharp, 1972). The current results extend our previous studies using DA-specific agents and an enkephalin analogue. Other neurotransmitters (norepinephrine, serotonin) also exert a definite, but less marked modulatory influence on the response of mice to morphine (Carroll and Sharp, 1972). The present findings provide behavioral support for a number of earlier suggestions, based upon anatomical and histochemical criteria, that the enkephalin and DA systems share a close functional relationship.

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