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Descending Systems Activated by Morphine (ICV) Inhibit Kainic Acid (IT)-Induced Behavior

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DeLANDER, G. E. AND J. J. WAHL. *Descending systems activated by morphine (ICV) inhibit kainic acid (IT)-induced behavior*. PHARMACOL BIOCHEM BEHAV **39**(1) 155–159, 1991.—Modulation of spinal systems activated by N-methyl-D-aspartate (NMDA) and substance P administered IT have been an area of interest in several laboratories. In the present investigations, behavior induced by the excitatory amino acid kainic acid, but not quisqualate, is demonstrated to be modulated in a manner similar to that previously observed for NMDA. Biting, scratching and licking behavior was induced by IT injections of excitatory amino acids or substance P in mice. Behavior induced by kainic acid (IT) injection was inhibited in a dose-dependent manner by coadministration of morphine (ICV), norepinephrine (IT), N-ethyl carboxamidoadenosine (NECA) (IT) and agonists interacting at PCP receptors (IT). Kainic acid and NMDA differed, however, in that a dopaminergic agonist, apomorphine, inhibited kainic acid-, but not NMDA-induced behavior and a selective NMDA receptor antagonist inhibits NMDA-, but not kainic acid-induced behavior. Behavior induced by quisqualate (IT) was not inhibited by any treatment and may have nonspecific actions in this type of assay. Our observations support independent spinal sites of action for behavior induced by kainic acid and NMDA, but several similarities were observed in the modulation of spinal systems activated by these agents.

Excitatory amino acids Spinal Kainic acid NMDA Quisqualate Substance P Morphine Adenosine Norepinephrine Dopamine PCP receptors

INVESTIGATIONS in our laboratory (8, 10, 11) and others (1, 2, 4, 5, 12, 13, 19) have examined potential interactions between putative spinal neurotransmitters and a variety of agents that induce antinociception when administered intrathecally (IT) in mice or rats. These studies have largely centered around agonists for substance P and NMDA receptors, since these receptors have been previously proposed as mediators of pain transmission. Investigations using IT injections of agonists or antagonists find that norepinephrine, serotonin and adenosine inhibit either substance P- or NMDA-induced behavior (1, 2, 8). Interactions with spinal mu opioid receptors have been observed to inhibit NMDA-induced behavior in mice and rats (2,19), but the interactions of opioids with substance P are less clear (4, 10, 17).

Our laboratory has established a functional link between morphine (ICV) activation of descending spinal systems and inhibition of behavior induced by NMDA or substance P administered IT (10). Morphine (ICV)-stimulated descending spinal systems that release spinal norepinephrine and adenosine, in particular, effectively inhibit NMDA and substance P-induced behavior. In the course of our investigations, we found preliminary evidence to suggest that interactions at other excitatory amino acid receptors types might also be influenced by neuromodulators that induce antinociception at spinal sites. Recent investigations by Kellstein et al. (19) suggest that, in rats, kainic acid receptor interactions appear to be modulated by opioids acting at mu and sigma receptors. Our present investigations demonstrated that, like NMDA receptors, effects mediated via interactions at spinal kainic acid receptors, in mice, were also inhibited by morphine (ICV), norepinephrine (IT) and adenosine analogs (IT). Inhibition mediated by these agents, however, did not generalize to inhibit behavior induced by IT administration of quisqualate. Preliminary results from some experiments have been reported previously (9).

METHOD

Animals

Male, Swiss-Webster mice (Simonsen; Gilroy, CA) weighing 20–30 g were used for all experiments. Animals were kept on a 12-hour light/dark schedule and allowed free access to food and water.

Experimental Protocol

Observation of licking, biting and scratching behavior induced by IT injection of excitatory amino acids or substance P was used to assess the efficacy of drugs administered IT. The assay involved injecting mice IT with glutamate (100 nmol), quisqualate (1 nmol), N-methyl-D-aspartate (NMDA, 0.25 nmol), kainic acid (60 pmol) or substance P (6 pmol). Animals were placed in an observation chamber immediately following injection and the number of caudally directed licks, bites or scratches made with the hind limbs were counted for a one-minute period. The dose

of each agent used was chosen because each caused comparable behavior (typically 70–90 licks, bites or scratches during the first minute following injection). Further, these doses did not cause vocalization and behavior was complete within approximately three minutes after injection. The mean number of behaviors, and standard error, observed in 8–10 mice was determined for each excitatory amino acid and used as a control value.

The efficacy of drugs as inhibitors of behavior was determined by coadministering the agent of interest with an excitatory amino acid or substance P IT. The effect of morphine was determined by administering morphine ICV 15 min prior to excitatory amino acid injection IT. The average number of licks, bites or scratches observed in mice in the treatment group was compared to behavior in control animals determined on the same day. Significant differences between mean number of licks, bites and scratches after treatments were determined using nonparametric analysis. The Mann-Whitney test was used to compare two means, while comparison of multiple means to a common control was performed with Friedman's test. Both tests were performed with the aid of the Prophet computer system. ED_{50} values, and 95% confidence intervals, were determined from dose-response curves with the aid of a computer (Pharm Basic. pcs; Life Sci. Assoc., Bayport, NY).

Drugs and Drug Administration

All drugs were administered in a total volume of 5 μl and dissolved in 0.9% saline, with the exception of haloperidol. Haloperidol was dissolved in absolute ethanol and then diluted with saline acidified with acetic acid (pH 3). The final injection vehicle contained 5% ethanol and had no effect on behavior when injected alone or on control values for excitatory amino acid-induced behavior. Intrathecal injections were made using modifications of the method of Hylden and Wilcox (16) that allow IT administration through intact skin and ICV injections followed the method of Haley and McCormick (14).

Norepinephrine HCl, haloperidol, apomorphine, quisqualic acid, kynurenic acid and kainic acid were purchased from Sigma Chemical Company (St. Louis, MO). L-Glutamic acid and NMDA were purchased from Aldrich Chemical Company (Milwaukee, WI). Substance P, [D-Pro^2-D-Tryp^{7,9}]substance P, morphine sulfate and NECA were products of Cambridge Research Biochemicals (Cambridge, England), Bachem Inc. (Torrance, CA), Mallinckrodt Chemical Works (St. Louis, MO) and Boehringer Mannheim (Mannheim, West Germany), respectively. The authors are grateful for generous gifts of (±)-5-methyl-10,11-dihydro-5H-dibenzo(a,d) cycloheptene-5,10-imine maleate (MK-801) (B. Clineschmidt, Merck, Sharp and Dohme Research Laboratories; West Point, PA), 2-amino-7-phosphono heptanoate (AP7) (P. Contreras, Searle Research and Development; St. Louis, MO) and phencyclidine (T. F. Murray, Oregon State University; Corvallis, OR).

RESULTS

Morphine administered ICV inhibited behavior induced by kainic acid administered IT in a dose-dependent manner (Fig. 1), but failed to significantly inhibit behavior induced by IT injection of either quisqualic acid or glutamate at doses up to 1.5 nmol morphine sulphate (ICV). The ED_{50} value of morphine (ICV)-mediated inhibition of kainic acid-induced behavior was 30.4 pmol (95% confidence interval = 10.0–91.9 pmol), indicating a potency comparable to that previously reported for NMDA-, and greater than that reported for substance P-induced behavior (11).

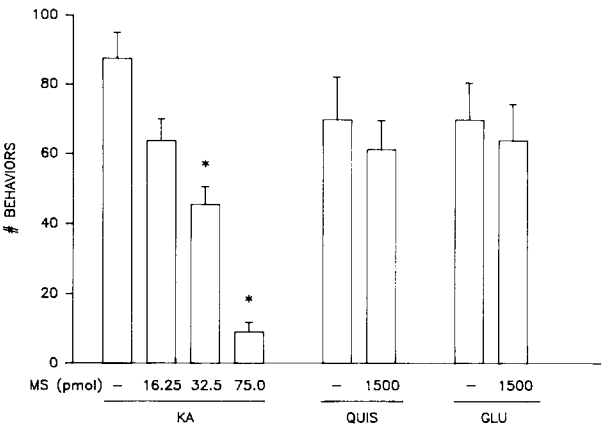

FIG. 1. Inhibition of excitatory amino acid-induced behavior by morphine. Mice were administered morphine ICV 15 min before IT injection of kainic acid (KA), quisqualic (QUIS) or glutamate (GLU). The number of caudally directed bites, scratches or licks were counted for a one-minute period immediately after IT injection and compared to animals that received saline ICV [n = 10 for each treatment group; S.E. are displayed; (*) significantly different from control, $\alpha = 0.01$].

The efficacy of norepinephrine (IT) (Fig. 2) and N-ethyl-carboxamidoadenosine (NECA) (Fig. 4) as inhibitors of behavior induced by various excitatory amino acids and substance P followed a profile similar to that observed with morphine (ICV). NECA inhibited kainic acid (IT)-induced behavior in a dose-dependent manner (Fig. 4), but quisqualic acid-induced behavior was not significantly inhibited (data not shown). Previous studies have demonstrated NECA (IT) administration to inhibit behavior induced by coadministration of NMDA and substance P, but not glutamate (8). Norepinephrine (IT) administration inhibited NMDA-, KA- and substance P-induced behavior, but unlike morphine (ICV), norepinephrine inhibited behavior induced by glutamate (IT). Inhibition of glutamate-induced behavior was significant, but inhibition was to a lesser extent than observed

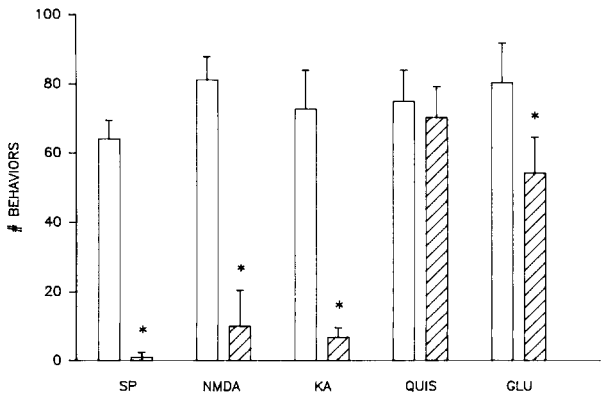

FIG. 2. Inhibition of excitatory amino acid- and substance P-induced behavior by norepinephrine. Mice were coadministered 100 pmol norepinephrine (IT) with NMDA, kainic acid (KA), quisqualic acid (QUIS), glutamate (GLU) or substance P (SP). The number of caudally directed bites, scratches or licks were counted for a one-minute period immediately after IT injection and compared to animals not administered norepinephrine [n = 10 for each treatment group; S.E. are displayed; (*) significantly different from control, $\alpha = 0.01$].

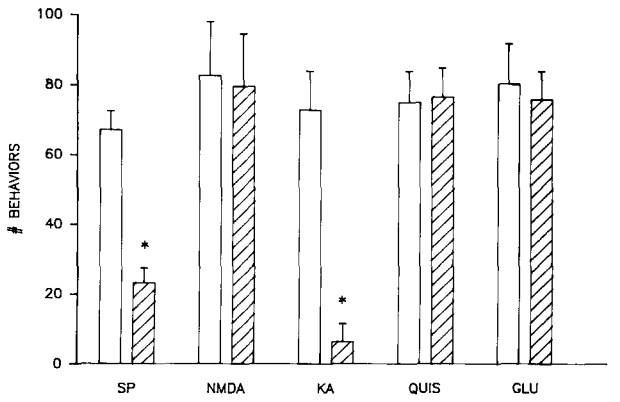

FIG. 3. Inhibition of excitatory amino acid- and substance P-induced behavior by apomorphine. Mice were coadministered 10 nmol apomorphine (IT) with NMDA, kainic acid (KA), quisqualic acid (QUIS), glutamate (GLU) or substance P (SP). The number of caudally directed bites, scratches or licks were counted for a one-minute period immediately after IT injection and compared to animals not administered apomorphine [n = 10 for each treatment group; S.E. are displayed; (*) significantly different from control, $\alpha = 0.01$].

for kainic acid, NMDA or substance P.

Kainic acid (IT)-induced behavior was also inhibited by phencyclidine receptor agonists, phencyclidine and MK-801 (Fig. 4). In contrast to results previously noted for substance P (11), inhibition of kainic acid-induced behavior by phencyclidine was not reversed by coadministration of haloperidol (1 nmol), a dopamine receptor antagonist (data not shown). Apomorphine, a dopaminergic agonist, coadministered IT, however, inhibited both substance P (IT)- and kainic acid (IT)-induced behavior, while having no significant effect on behavior induced by IT injection of NMDA, quisqualate or glutamate (Fig. 3). The selective NMDA receptor antagonist, 2-amino-4-phosphonoheptanoic acid (AP7, 20 nmol), and the selective tachykinin receptor antagonist, D-Pro^2-D-Tryp^{7,9}-substance P (1 nmol), were ineffective as inhibitors of kainic acid (IT)-induced behavior (data not shown).

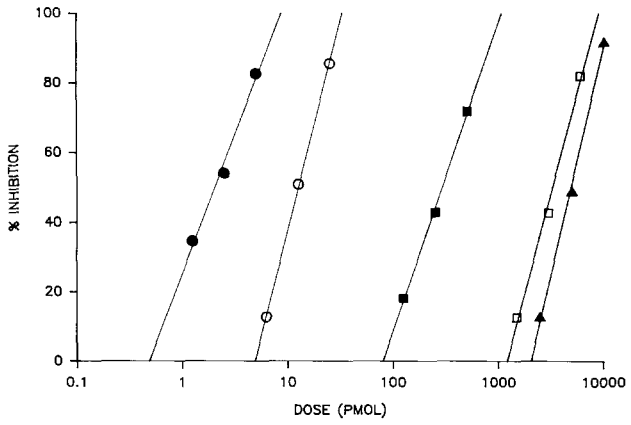

FIG. 4. Dose-response curves for inhibition of kainic acid-induced behavior by compounds coadministered IT. Mice wre coadministered kainic acid with various doses of NECA (●), norepinephrine (○), MK-801 (■), PCP (□) or apomorphine (▲). The number of caudally directed bites, scratches or licks were counted for a one-minute period immediately after IT injection and expresses as a percentage of behaviors observed in animals administered only kainic acid. (n = 10 for each treatment group.)

DISCUSSION

Morphine administered ICV causes antinociception via direct and indirect mechanisms. Indirect mechanisms are thought to include the activation of descending spinal systems that inhibit nociceptive transmission at a spinal level. Recent investigations in our laboratory establish a functional link between morphine (ICV) activation of spinal systems and the inhibition of behavior induced by putative pain neurotransmitters interacting at substance P and NMDA receptors (11). Spinal systems releasing norepinephrine or adenosine appear to be the most likely mediators of these indirect effects of morphine administration. Our observations are supported by a variety of studies that use agonists and antagonists administered IT to suggest that norepinephrine (20, 26–28) and adenosine (3, 6, 7, 10, 11, 24) are likely mediators of spinal effects induced by morphine (ICV) administration.

Investigations performed in our laboratory suggested that administration of morphine (ICV), and some spinally active antinociceptive agents, may also modulate effects elicited by interactions at non-NMDA receptors. Kainic acid (IT)-induced behavior, in particular, appeared to be altered by several agents in a manner similar to that observed for NMDA-induced behavior. Behaviors induced by IT administration of kainic acid were inhibited by coadministration of morphine (ICV), norepinephrine (IT) and NECA (IT). Potency for inhibition of kainic acid-induced behavior was comparable to that for inhibition of NMDA- or substance P-induced behavior, although each agent was slightly more potent against kainic acid.

Kainic acid receptors, like NMDA and substance P receptors, are concentrated in lamina II of the spinal cord (18). Our data suggests that secondary spinal neurons stimulated by neurotransmitter interactions at NMDA, kainic acid or substance P receptors may be modulated by common descending spinal systems which can be activated by morphine (ICV) administration. In spite of several similarities observed in the modulation of NMDA and kainic acid, NMDA and kainic acid receptor interactions appear to be independent. A selective NMDA receptor antagonist, AP7, was ineffective as an inhibitor of kainic acid (IT)-induced behavior at the doses tested and kainic acid-, but not NMDA-induced behavior was inhibited by coadministration of apomorphine (IT), a dopaminergic agonist. Further, failure of a selective tachykinin receptor antagonist, DPDPT, to inhibit kainic acid-induced behavior supports independent neuronal mechanisms for kainic acid and substance P.

Independence of NMDA- and kainic acid-mediated actions is difficult to reconcile with inhibition of kainic acid-induced behavior by agents characterized as noncompetitive NMDA receptor antagonists, PCP and MK-801. In the present study, kainic acid-induced behavior was inhibited by coadministration of PCP or MK-801, although both agents were less potent inhibitors of kainic acid- than NMDA-induced behavior. Some similarities in the regulation of NMDA and kainic acid receptor-mediated actions have also been observed in reports from other laboratories (15, 19, 21, 23). Inhibition of kainic acid-induced toxicity by MK-801 led Michaels and Rothman (21) to suggest that kainic acid may be able to nonspecifically open NMDA channels. It would be difficult to make predictions of this type based on our results, but similarities in regulation may not be wholly unexpected since interactions at both kainic acid and NMDA receptors are reported to activate voltage-dependent calcium channels as at least part of their mechanism of action (22).

Conflicting reports of the regulation of kainic acid receptor-

mediated actions have been noted. Aanonsen and Wilcox (1,2) used protocols very similar to those used by our laboratory with mice and by Mayer and co-workers (13,19) with rats. Aanonsen and Wilcox (1,2) demonstrated modulation of NMDA-induced behaviors by coadministration (IT) of opioids, norepinephrine or PCP in mice, but found no evidence to support similar regulation of kainic acid-induced behavior in mice. Kellstein et al. (19) suggest that differences between their observations in rats and those of Aanonsen and Wilcox (1,2) in mice may be accounted for by variations between species, but our investigations using mice were also inconsistent with the observations of Aanonsen and Wilcox (1,2).

Differences between reports may actually reflect a dose-dependent phenomena. Doses of NMDA and kainic acid chosen to induce 70–90 licks, bites or scratches following IT injection for the present investigations were 250 pmol and 60 pmol, respectively. Aanonsen and Wilcox (2) used equal doses of 250 pmol NMDA or kainic acid to induce 30–35 behaviors. It is difficult to determine why such a wide difference in doses and behavior is observed in these two studies. Injections of 250 pmol kainic acid (IT) in our laboratory (data not shown) induced a near convulsive state with significant changes in each mouse's posture. The approximately four-fold difference in the potency of NMDA and kainic acid observed in our studies is similar to the difference in potency noted in separate investigations by Urca and Raigorodsky (25). Therefore, the failure of opioids, norepinephrine and PCP agonists to inhibit kainic acid-induced behavior in the studies of Aanonsen and Wilcox (2) may be explained by nonspecific effects of kainic acid at the doses used.

Nonspecific actions of kainic acid at high doses may be similar to the actions induced by quisqualate administration (IT). In the present study, similarities observed for regulation of NMDA and kainic acid receptor-mediated actions were not shared by quisqualate receptors. Behavior induced by interactions at quisqualic acid receptors was distinguished from NMDA- and kainic acid-mediated actions by the failure of any compounds tested to inhibit quisqualic acid-induced behavior. In addition to the agents noted previously, coadministration of kyenurate (IT), a nonselective excitatory amino acid receptor antagonist that inhibited behaviors induced by IT injections of both NMDA and kainic acid in our laboratory, failed to inhibit quisqualate-induced behavior (data not shown). These agents also failed to inhibit glutamate-induced behavior, with the exception of a modest norepinephrine-mediated effect at the highest dose tested. The large doses of quisqualic acid (1 nmol) and glutamate (100 nmol) required to reliably induce behavior and the failure of kyenurate to inhibit behavior induced by these two compounds suggests that their actions in this type of assay are nonspecific.

These investigations extend our attempts to characterize neuronal transmission at spinal sites. Injections of NMDA, kainic acid or substance P (IT) elicited similar behaviors presumably by interacting with their respective receptors on secondary spinal neurons. Results observed following coadministration of selective receptor antagonists suggested that independent receptor interactions mediate the actions of each agonist. Recent studies by Hornfeldt and Larson (15) using IT administered cations confirm our characterization of distinct quisqualic acid, kainic acid and NMDA receptors at spinal sites in the mouse. Although independent afferent systems are described, it appears that these systems may be inhibited or regulated by common descending spinal systems. Morphine administered ICV is known to activate descending pathways that can release norepinephrine and adenosine spinally to inhibit spinal nociceptive transmission. Our investigations demonstrated that morphine (ICV) inhibited behaviors elicited by NMDA, kainic acid and substance P administered IT and morphine (ICV)-induced inhibition was mimicked by IT administration of norepinephrine or adenosine.

The physiologic significance of similarities in the regulation of behaviors induced by NMDA, kainic acid and substance P is difficult to determine at this time. It may indicate that unique afferent systems activated by distinct stimuli are regulated in a common manner or it may simply reveal a redundancy in systems that allow animals to respond to environmental stimuli in general. Similarly, based on current knowledge, it is difficult to explain mechanisms responsible for inhibition of kainic acid-induced behavior by noncompetitive NMDA receptor antagonists. Future research in this field, however, can be expected to reveal a complete functional characterization of spinal afferent systems.

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