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Research report

Multiple neurotransmitter receptors contribute to the spinal Fos expression

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Abstract

The aim of this study is to identify the receptors which could potentially mediate the activation of c-Fos. Therefore, the effects of neurotransmitter receptor agonists in the activation of c-Fos in spinal neurons were studied by intrathecal injection of excitatory amino acid (EAA) receptor agonists: *N*-Methyl-D-Aspartate (NMDA), (*S*)-α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic acid (AMPA), 2-Carboxyl-3-carboxmethyl-4-isopropenylpyMidine (Kainic acid, KA), (1S-3R)-1-Aminocyclopentane-1, 3-dicarboxylacid (ACPD), and substance-P receptor (neurokinin-1) agonist, [Sar^9, Met $(O_2)^{11}$] SP (SarMet-SP). All drugs tested activated the production of c-Fos in spinal dorsal horn neurons. AMPA was found as the most potent agonist tested producing market production of c-Fos particularly in neurons of lamina II at doses of 10 pM per 10-μl injection. At this dose, other agonists were relatively ineffective. At higher doses, AMPA significantly increased the activated cells. NMDA significantly increased c-Fos production to a marked extent only at doses above 10 nM per 10-μl injection. KA and ACPD were least potent of the excitatory amino acid agonists. Injection of SarMet-SP at doses of 1 nM activated Fos selectively in neurons of lamina I. A dose-dependent increase in number of c-Fos-positive cells was observed for AMPA, KA, ACPD, and SarMet-SP, whereas NMDA gave a very strong expression after a high dose with no dose dependency. These finding suggest that multiple neurotransmitter receptors lead to c-Fos production in spinal neurons.
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Theme: Neurotransmitter, modulators, transporters, and receptors
Topic: Excitatory amino acid receptors: structure, function, and expression

Keywords: NMDA; AMPA; Kainic acid; ACPD and SP receptors; c-Fos; Intrathecal injection; Spinal cord

1. Introduction

Excitatory amino acid (EAA) receptor subunits [14, 38,57] and Substance P receptor [20,28] are expressed in the spinal cord. Cloning and sequencing of EAA receptors and tachykinin receptor subtypes have been done (e.g., [21,51], respectively). A range of selective agonists for these receptors is available.

The function of glutamate as an excitatory transmitter for primary afferent neurons is well established. For example, mediation of excitatory synaptic responses by NMDA and AMPA receptors in nociceptive neurons after noxious stimulation [19], producing of nociceptive action following intrathecal application of EAA agonists to the subarachnoid space in mice and rats [1], and reducing noxious chemical stimuli-induced nociceptive actions by intrathecal application of EAA antagonists in mice [37] and in rats [45,46] have been demonstrated. Wilcox [59] has reviewed more evidence for the potential role of EAA in nociception. SP has been described as "a neurotransmitter for pain" [40]. It has been demonstrated that nociceptive behavior is observed following SP injection to the subdural space [15,27,49], and a selective antagonist to NK1 receptor reduces nociceptive action induced by NK1 receptor agonist [39].

Demonstration of c-Fos that is the protein product of the c-*fos* gene in neurons of the dorsal horn of the spinal cord following noxious cutaneous stimuli [24] immediately stimulated considerable research. This is because c-Fos could be used as an activity marker to map pathways activated by specific stimuli, drugs, and behavioral changes [13,33,48]. In the field of pain, it offered a method to identify the neurons

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activated by noxious stimuli and thus identify nociceptive pathways [10,18,24,30]. It also permitted the investigation of the effects of analgesic drugs such as morphine on the activation of these pathways [4,16,17,41,57].

Several reports have emerged demonstrating neurotransmitter induced-c-*fos* expression in brain [12,35,52,55,58] and in spinal cord [6]. Some antagonist experiments have demonstrated that glutamate agonists are involved in c-Fos expression in the spinal cord in response to sensory stimulation [7,26,54]. In the spinal cord, what type of neurotransmitter receptors activates the production of c-Fos has not been fully demonstrated by intrathecal agonist experiments. Therefore, the effects of neurotransmitter receptor agonists in the activation of c-Fos in spinal neurons were studied by intrathecal injection of excitatory amino acid (EAA) receptor agonists: *N*-Methyl-D-Aspartate (NMDA), (*S*)-α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic acid (AMPA), 2-Carboxyl-3-carboxmethyl-4-isopropenyl-pyMidine (Kainic acid, KA), (1S-3R)-1-Aminocy-clopentane-1, 3-dicarboxylacid (ACPD), and substance-P receptor (neurokinin-1) agonist, [Sar^9, Met $(O_2)^{11}$] SP (SarMet-SP).

Related preliminary report has been made of some of the immunocytochemical data reported by Soygüder and Morris [53].

2. Materials and methods

2.1. Animals

Adult 42 Wistar rats of either sex were used in this study. Large adult rats (over 300 g) were employed to reduce possible canula damage. All rats were obtained from the breeding stock in the Veterinary Pathology Department, University of Liverpool. They were housed in standard cages (North Kent Plastics Ltd, Home Gardens, Dartford) and in animal rooms at 20 °C with lighting for 12 h each day and 55% humidity. Animals were maintained with food and water ad libitum.

Due to the possibility that stress and sensory stimulation could possibly lead to c-*fos* induction, stress and animal handling was minimized. The animals were transported from the animal house in a cage enclosed in a basket to reduce arousal. Prior to sensory stimulation, they were kept in separate cages and handling was kept to a minimum. The animals were supplied with water. Care was taken to minimize the effects of environmental variables such as room temperature and noise.

2.2. Anesthesia

Anesthesia was induced with halothane (Fluothane, Zenica) 4% in air and then long-term stable anesthesia established with ethyl carbamate (Urethane, Sigma) at a dose of 1000 mg/kg IP. Further doses of urethane (100 mg/kg) IP were administered if the depth of anesthesia becomes shallow as assessed by increase in respiratory rate or a reflex withdrawal to a pinch stimulus. Pinches used to assess depth of anesthesia were employed as little as possible and only applied to the fore limbs to avoid induction of c-*fos* to these stimuli. Following anesthesia, the animals were kept warm on a thermostatically controlled heating blanket and covered with cotton wool. This procedure was designed to maintain skin temperatures constant between animals. Prior to vascular perfusion, an overdose of anaesthetic (Sagatal, pentobarbitone-sodium, May and Marker Ltd, 50–60 mg/kg IP) was given to ensure very deep anesthesia during the aggressive surgery involved in this stage of the protocol.

2.3. Drugs

Four selective agonists for excitatory amino acid receptors and one tachykinin receptor agonist were administered intrathecally in this study. Drugs were tested at various concentrations selected on the basis of their actions reported in pharmacological and behavioral studies (e.g., [37,46,49]). Selective agonists were chosen to activate sub-groups of ionotropic EAA receptors. These were *N*-Methyl-D-Aspartate (NMDA, Sigma), (*S*)-α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic acid (AMPA, Tocris), and 2-Carboxyl-3-carboxmethyl-4-isopropenylpyMidine (Kainic acid, Calbiochem). A selective agonist (1S-3R)-1-Amino-cyclopentane-1, 3-dicarboxylacid (1S, 3R-ACPD, Tocris) of metabotropic glutamate receptors and a tachykinin receptor agonist [Sar^9, Met $(O_2)^{11}$] SP (SarMet-SP, Nova) which is selective for NK_1 receptors were also used.

2.4. Intrathecal injection

A simplified version of the method described by Yaksh and Rudi [60] was used. A polythene tubing (ID 0.28 m, OD 0.61 m, Portex 800/100/100) was inserted into another wider catheter for about 1 cm and joined with glue (Loctite Prism 406). The thin catheter was cut at 7.5 cm length from the distal tip of the wider one. On the catheter, the distal tip of the wider one was marked by a black marker pen. This mark was used as to indicate when catheter had been inserted the appropriate distance in the subdural space. Then the distal end of the thin canula was introduced into the subdural space via the hole in the atlanto-oxipital membrane and gently inserted until its tip reached the lower thoracic level as indicated by the mark on the canula. Following the cannulation, freshly prepared drug solutions, in ACSF, were injected with a 10 μl Hamilton syringe in a volume of 10 μl, followed by 10 μl of normal saline to wash the drug through the canula. First drug injection was made 10 min after the cannulation. Four injections of the same drug or vehicle were made at 30-min intervals. Animals were perfused 30 min after the last drug injection. Control animals received equivalent vehicle injections in the same manner.

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