ATP-sensitive potassium channels and endogenous adenosine are involved in spinal antinociception produced by locus coeruleus stimulation

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Abstract It has been known that locus coeruleus (LC) stimulation suppresses nociceptive discharges of the thalamic parafascicular (PF) neurons through the spinally descending adrenergic terminals which inhibit the transmission of nociceptive signals in the spinal dorsal horn. This experimental model was used in the present study to analyze the detailed processes that happened in the dorsal horn following norepinephrine release by preemptive intrathecal i.t. administration of related drugs in lightly urethane-anesthetized rats. The results showed that LC stimulation significantly inhibited the noxiously-evoked discharges of PF neurons, the LC stimulation-produced antinociception in PF neurons could be blocked either by i.t. glibenclamide an ATP-sensitive potassium (K<sub>ATP</sub>) channel blocker or by i.t. aminophylline an adenosine receptor antagonist. Nociceptive discharges of PF neurons were also suppressed both by i.t. 5'-N-ethylcarboxamido-adenosine, an adenosine receptor agonist and by i.t. nicorandil, a K<sub>ATP</sub> channel opener and 4(1)-i. t. aminophylline blocked the suppression of PF nociceptive discharges induced by i.t. nicorandil while i.t. glibenclamide showed no effect on the suppression of nociceptive discharges induced by i.t. NECA. These results suggest that K<sub>ATP</sub> channels and endogenous adenosine may be involved in the mediation of spinal antinociception induced by descending adrenergic fibers originating from the LC and to the opening of K<sub>ATP</sub> channels precedes the release of adenosine in the cascade of mediation.

Key words locus coeruleus stimulation; parafascicular nociceptive neurons; intrathecal injection; ATP-sensitive potassium channel; adenosine

Norepinephrine (NE) is one of the neuroactive substances involved in the modulation of nociceptive input at the spinal level. Locus coeruleus (LC) neurons are mostly norepinephrinergic (NE-ergic) and the...
descending NE-ergic fibers originating from LC suppress nociceptive transmission in the spinal dorsal horn [16]. Other neuromodulators such as opioids [13] and adenosine [17] and ATP-sensitive potassium [18] K+ ATP channels have also been considered to be involved in the modulation of spinal nociceptive transmission. In a series of behavioral studies we have proposed that NE molecules either released in the dorsal horn from the descending terminals or administered exogenously exhibit suppressive effects on spinal nociceptive transmission through a multiple cascade comprising endogenous opioids K+ ATP channels and adenosine in a sequential order [10,14].

Therefore the objective of the present study was to elaborate further the above proposal by using electrophysiological techniques in rats in which nociceptive discharges were evoked peripherally and recorded from the medial thalamus-parafascicular PF neurons while the LC was stimulated in combination with intrathecal administration of some relevant drugs. This is based on the fact that we have previously demonstrated that LC stimulation inhibits the nociceptive discharges in PF neurons indirectly through the suppression of nociceptive transmission at the spinal level by way of descending NE-ergic terminals [19].

With this experimental model we intended to elaborate what happened in the spinal circuit following the release of NE. In the present study only the involvement of K+ ATP channel and adenosine as well as their relation in the cascade was assessed.

1 MATERIALS AND METHODS

Forty-eight Wistar rats of both sexes weighing 170240 g were used. Rats were anesthetized with urethane [1] 1.25 g/kg i.p. and an i.t. catheter was implanted according to the procedure described by Yaksh and Rudy [15]. Polyethylene tubing PE-10 was filled with sterile normal saline NS was inserted through a small incision on the atlanto-occipital membrane and extended 7.5 cm caudally to the lumen enlargement of the spinal cord. Subsequently the rat’s head was secured in a stereotaxic frame and two 1-mm-diameter holes were drilled on the left side of the skull for inserting electrodes into the PF and LC. A glass micro pipette filled with 0.5 mol/L sodium acetate and 2% pontamine sky blue was used for extracellular single-unit recording. The ohmic resistance of the electrode ranged from 10 to 30 MΩ. The recording microelectrode was inserted into the PF area according to the coordinates of Paxinos and Watson [16] P 4.04 3 L 0.91 2 H 4.56 5. The signals from the recording electrode were led to an amplifier SEZ-8201 displayed on an oscilloscope and processed online with a desktop computer. For LC stimulation a bipolar twisted electrode made of stainless steel wires insulated except at the tip 80 μm in diameter was inserted into the LC area P 9.39 8 L 1.21 5 H 6.77 5 [16] and was fixed to the skull with dental cement. The stimulation parameters were 0.3 ms square pulse 100 Hz 0.2 mA and 15 s in duration.

Noxious responses of PF neurons were elicited by high-intensity electrical stimulation 0.3 ms square pulse 100 Hz 2.0 to 3.6 mA and 25 trains applied to the contralateral sciatic nerve [12,30]. In addition the nociceptive properties of responses were confirmed by their similarity to those elicited by natural pain-inducing procedures tail pinch [17].

Aminophylline Aph an adenosine receptor antagonist [10] Shanghai Xinyi Pharmaceuticals China 5′-N-ethylcarboxamido-adenosine NECA an adenosine receptor agonist Sigma d nicorandil Nico an opener of K+ ATP channel Datong Pharmaceuticals China were freshly prepared with NS and glibenclamide Gli a blocker of K+ ATP channel Sigma was dissolved with 5% Tween 80 in NS. Each drug was injected slowly 1 min in a volume of 10 μl followed by 10 μl NS to flush the catheter of its contents. The dosages used in the present experiments were determined by dose-response analyses in previous behavioral studies [11–14]. Experimental observations began about 2 h after surgery. Once single unit discharges were obtained from PF area they were monitored for 3 to 5 min to verify their stability of spontaneous discharges. When stable baseline was obtained the following protocol of recordings was used [1] I 1 min of spontaneous discharges followed by sciatic stimulation and 3 min recording of noisily-evoked discharges 2 10 s of spontaneous discharges followed by 15 s stimulation of LC and immediately after which the noxious stimulation was applied as in I and followed by 3 min recording of nociceptive discharges [3] 5 min after i.t. Aph or Gli administration procedures 1 and 2 were repeated several times with 5 min intervals [4] 5 min after i.t. NECA or Nico procedure 1 was repeated several times with 5 min intervals and [5] 5 min after i.t. co-administration of
NECA + Gli or Nico + Aph procedure 1 was repeated several times with 5 min intervals.

Noxiously-evoked changes in discharges were calculated for each neuron and expressed as the percentage changes against the baseline spontaneous discharges before sciatic stimulation by the following formula[4] percentage change in discharges evoked by noxious stimulation \( n = B - A / A \times 100\% \) where \( A \) is the total number of spontaneous firings in 10 s before the sciatic stimulation and \( B \) the total number of firings in 10 s immediately after the end of sciatic stimulation. When evaluating the effects of LC stimulation on the nociceptive responses[1] the noxiously-evoked percentage changes obtained in conditions with or without preceding LC stimulation were compared. When evaluating the effects of drugs on the LC stimulation-induced effects[4] the LC stimulation-induced percentage changes in nociceptive discharges obtained in conditions with or without pretreatment of i.t. drug administration were compared for each neuron and then compared among different neuronal groups. All data were expressed as the mean ± SE. Differences between groups were analyzed using the two-tailed Student’s test and ANOVA.

At the end of each experiment[1] pontamine sky blue was injected iontophoretically through the recording electrode to mark the last recording site in PF. Two mA DC current was delivered via the LC electrode for 10 s to produce a small lesion. Recording sites in PF and stimulation sites in LC were histologically identified in 50 μm frozen sections.

2 RESULTS

2.1 Effect of noxious stimulation

Sixty-three PF cells with stable baseline were recorded following noxious sciatic electrical stimulation. As reported previously[1-3] the noxiously evoked discharges recorded in PF area exhibited three types of response that could be classified as “nociceptive-on” \( n = 48 \) 76% \( \% \) nociceptive-off \( n = 9 \) 15% \( \% \) or “non-responsive” \( n = 6 \) 9% cells. Only 38” nociceptive-on” cells simply named as “nociceptive” later \( \% \) cells were used for further observation.

2.2 Effects of LC stimulation

Nociceptive discharges in 20 PF units were recorded combined with preceding LC stimulation. Histograms in Fig. 1A and also in Fig. 2A present frequency density histograms obtained before column I and after column II LC stimulation in a representative neuron. As summarized in columns I and II in Fig. 1B and Fig. 2B[4] the nociceptive discharges were suppressed by LC stimulation in all the 20 PF neurons[4] with a mean suppressive rate of 84.1 ± 17.7% \( \% \) \( P < 0.001 \) as compared to the control.

Fig. 1. A] Representative frequency histograms of a parafascicular PF nociceptive neurons showing the nociceptive discharges \( \% \) the nociceptive discharges immediately after locus coeruleus \( \% \) LC stimulation \( \% \) II \( \% \) II \( \% \) the nociceptive discharges after i.t. aminophylline[4] Aph \( \% \) 120 nmol \( \% \) II \( \% \) II \( \% \) and the nociceptive discharges immediately after LC stimulation in the presence of i.t. Aph[4] \( \% \) II \( \% \) all the stimulation artifacts are not shown \( \% \) bin width \( \% \) 1 s. B] A summary of 10 PF nociceptive units tested with similar protocol as in A[4] the nociceptive discharges of each neuron was arbitrarily set as 0% for control of this neuron in order to estimate the percentage changes in the following observation. ↑ indicates the time of noxious stimulation[4] indicates the noxious stimulation with preceding LC stimulation.

2.3 Effect of i.t. aminophylline[4] Aph on LC stimulation-produced suppression of nociceptive discharges in PF neurons

Columns III and IV in Fig. 1A present frequency density histograms of a representative PF nociceptive neuron showing that i.t. Aph[4] 120 nmol[4] itself exerted no[4] if any effects on both the baseline and nociceptive discharges[4] column III as compared to column I[4] while the LC-induced suppressive effect on nociceptive discharges was blocked by Aph administration[4] column IV as compared to column II[4]. Figure 1B summarized the data in 10 nociceptive neurons tested by the same experimental protocol as in A[4] showing that Aph pretreatment failed to
change the baseline and nociceptive discharges column III as compared to column I but blocked the suppressive effect of LC stimulation column IV i.e. LC stimulation suppressed the nociceptive discharges with a weakened suppressive rate of 20.7 ± 23.9% column IV vs III P < 0.01.

2.5 Effect of i.t. nicorandil© Nico© on the nociceptive discharges in PF neurons and the blockage of it by i.t. aminophylline© Aph©

In order to elaborate if the opening of K+ATP channels precedes the release of adenosine in mediating the suppressive action of LC observed in the above two series of experiments© i.t. Nico© an opener of K+ATP channels© was used to initiate the suppressive action on nociceptive responses and the effect of i.t. Aph on it was then tested. Figure 3A presents the frequency density histograms obtained in a representative neuron© showing that the nociceptive discharges column III were mostly suppressed by pre-treatment of i.t. Nico© 0.3 nmol© 5 min prior to the noxious stimulation© column IV as compared to column III© meanwhile when i.t. Nico was given concomitantly with i.t. Aph© 120 nmol© the suppressive action of Nico on nociceptive discharges was largely blocked by Aph© column III as compared to II© Figure 3B summarizes the similar observations in 8 PF units© showing that i.t. Nico induced a mean decrease rate of 88.8 ± 11.2% in nociceptive discharges column III© while this decrease was 68.8 ± 15.7% in nociceptive discharges column II© with i.t. Aph© 120 nmol©
ceptive discharges\( \text{column I} \) as compared to \( \text{column II} \) while the mean decrease rate was only 19.8 ± 22.3% when Nico was co-administered with Apl\( \text{column III} \) vs \( \text{column II} \) \( P < 0.05 \).

3 DISCUSSION

The locus coeruleus\( \text{LC} \) is a brain stem nucleus composed mostly of norepinephrine-containing neurons that project fibers to different CNS areas\( \text{one of these projections is the spino-} \text{descending NE-ergic pathway} \text{which participates in the suppression of nociceptive transmission in the dorsal horn} \text{[15]}. \text{The thalamic parafascicular nucleus} \text{PF contains neurons that receive ascending nociceptive inputs from the spinal dorsal horn} \text{[17,19]}. \text{This means that peripherally-evoked nociceptive discharges of the PF neurons should have been processed at the relay station comprised of complex neuronal circuits in the spinal dorsal horn.}

Our previous studies have shown that LC stimulation suppresses nociceptive transmission at the spinal level by way of descending NE-ergic fibers through the activation of \( \alpha_2 \)-adrenergic receptors\( \text{resulting in a reduction of nociceptive discharges in PF} \text{[5]}. \text{Thus the present experimental procedures were designed to evaluate in detail the intricate processes emerging in the spinal dorsal horn by observing the changes of nociceptive discharges in PF} \text{following the LC stimulation combined with i.t. administration of related drugs} \text{as an experimental model as established and used previously} \text{[37,39,40]}. \text{The results in the present study have demonstrated that i.t. Apl significantly prevents the LC stimulation-induced suppression of the nociceptive transmission in the dorsal horn suggesting that adenosine is involved in the mediation of LC stimulation-induced antinociception at the spinal level} \text{in addition our experiments have demonstrated that i.t. Gli also blocks LC stimulation-induced spinal antinociception suggesting that both adenosine and K*}_{ATP} \text{channels are involved in the mediation of antinociception induced by NE that is released from LC descending NE-ergic fibers. Although only one dose of Apl} \text{120 nmol} \text{or Gli} \text{10 nmol} \text{was used in the present study these dosages have proved to be adequate to block the effects of related agonists by dose-response analysis conducted in previous behavioral studies} \text{[112]}. \text{Furthermore we have demonstrated that i.t. Gli fails to block the antinociception induced by i.t. NECA an adenosine receptor agonist while the antinociception induced by i.t. Nico an opener of K*}_{ATP} \text{chan-}
nals can be blocked by i.t. Aph. These results suggest that the opening of the K⁺ ATP channels may precede the release of endogenous adenosine in the cascade mediating the suppression induced by LC stimulation. Thus the present observation provides new support for the proposal developed previously from our behavioral and electrophysiological studies i.e. the NE molecules either released endogenously from the descending adrenergic fibers or exogenously administered into the dorsal horn may first promote the release of opioids from local opioi-dergic neurons not tested in the present study then the enkephalin peptide opens the K⁺ ATP channels through activation of α₂- and δ-receptors which may coexist with K⁺ ATP channels on the membrane postsynaptic to the opioi-dergic terminals and after that the process is followed by a release of endogenous adenosine. At this time however we still do not know exactly how the opening of K⁺ ATP channels promotes the release of adenosine and what the mechanism underlying the action of the adenosine is. It awaits further morphological and functional analysis on these problems.

REFERENCES


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