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SCIENCE DIRECT

European Journal of Pharmacology 512 (2005) 153–156

www.elsevier.com/locate/ejphar

Short communication

In vivo measurement of noradrenaline in the locus coeruleus of rats during the formalin test: A microdialysis study

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Received 10 January 2005; received in revised form 16 February 2005; accepted 22 February 2005
Available online 29 March 2005

Abstract

The locus coeruleus is involved in the regulation of the sense of pain. To demonstrate the changes in noradrenaline level in the locus coeruleus during the formalin test, a microdialysis probe was implanted into the left locus coeruleus of rats. Formalin was subcutaneously injected into the plantar surface of the right hind paw and pain ratings were recorded. The concentrations of noradrenaline and its metabolite 3-methoxy-4-hydroxyphenylethylenglycol (MHPG) were measured. The results showed an almost four-fold elevation in noradrenaline release in the early phase of the formalin test; levels return to baseline in the late phase. Levels of MHPG changed in a similar fashion.

Keywords: Microdialysis; Pain; Formalin test; Locus coeruleus; Noradrenaline

1. Introduction

The locus coeruleus is a bilateral pontine structure with widespread projections to numerous parts of the central nervous system (Fritschy and Grzanna, 1990). Noxious stimuli, such as foot shock as well as electrical stimulation of the locus coeruleus, are associated with an increase in locus coeruleus discharge and accelerated noradrenaline turnover in the cerebral cortex (Singewald et al., 1999). Pain induced by formalin in rodents has two phases, each reflecting different pathological processes (Shibata et al., 1989). Thus the locus coeruleus may have different roles in controlling the response in the formalin test.

One of the methods used to assess the locus coeruleus activity is in vivo assay of its chemicals by microdialysis (Davies et al., 2000). This technique has contributed significantly to our insight into the pharmacological properties of the noradrenergic locus coeruleus projections (Van Veldhuizen et al., 1990; Singewald and Philippu, 1998). Using a microdialysis technique, we measured the release of noradrenaline and its main metabolite, 3-methoxy-4-hydroxyphenylethylenglycol (MHPG), during different phases of the formalin test.

2. Materials and methods

2.1. Ethics

The protocol used in this study was approved by the Tarbiat Modarres Ethics Committee.

2.2. Animals

Six male albino Wistar rats (280–320 g) were used in each group for the experiments.

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doi:10.1016/j.ejphar.2005.02.032

2.3. Surgery and brain dialysis

2.3.1. Preparation and calibration of the dialysis probe

Concentric microdialysis probes with an active dialysis length of 1 mm were constructed in our laboratory from regenerated cellulose dialysis tubing (spectra/pro hollow fiber: molecular weight cutoff: 6000 Da; 0.250 mm OD (Sharp and Zetterstrom, 1992). The probes were tested in vitro to determine the relative recovery of noradrenaline and its main metabolite, MHPG, prior to implantation, as previously described (Robert et al., 1993); recovery was 25%.

2.3.2. Probe implantation

Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and the probe was implanted into the left locus coeruleus (A/P: −9.8 mm from the bregma, L/M: 1.2 mm D/V: −7.1 mm, vertically). Probes were perfused with artificial cerebrospinal fluid (ACSF) at a flow rate of 2.0 μl/min (WPI, SP 210, syringe pump) and fractions were collected every 15 min. The composition of ACSF was (in mM): NaCl 114, KCl 3, $CaCl_2$ 1, $MgSO_4$ 2, NaH_2PO_4 1.25, $NaHCO_3$ 26, NaOH 1, glucose 10, and pH=7.4.

2.4. Formalin test

The tests were carried out as described before (Dubuisson and Dennis, 1977). First, ACSF was perfused. After 1 h of sample collection, 50 μl of 2.5% formalin was subcutaneously injected into the plantar region of the right hind paw with a 27-gauge needle for noxious stimulation.

Pain rating was recorded according to the behavioral categories: 0, 1, 2 and 3 (Dubuisson and Dennis, 1977). Rats were observed for 60 min. The results of the formalin test are presented as mean of pain scores during the first 5 min (early phase) and 20–60 min (late phase) after formalin injection.

2.5. Chemical assays

Noradrenaline and MHPG were quantified by high-pressure liquid chromatography (HPLC) with electrochemical detection (ECD). A Waters 510 Pump (USA) was used in conjunction with an electrochemical detector (Pharmacia LKB, type-2143 RPE, USA) to measure the catecholamines. The oxidizing potential of the ECD cell was 750 mV. A reverse-phase column (Teknokroma, 120 ODSA, 150×4.6 mm) was used. The mobile phase consisted of a mixture of sodium phosphate 8.4 g, 1-octane-sulfonic acid 360 mg, EDTA 30 mg and 12% of methanol in 1000 ml H_2O (pH=4.6). The flow rate of mobile phase was 1.0 ml/min. The detection limit of the assay was 5 fmol per sample (on-column).

2.6. Expression of results and statistics

All values given are mean ± S.E.M. of the measured parameters. Data were analyzed by two-way repeated-measures analysis of variance. The level of significance was set at $P<0.05$. Student's t-test for unpaired data was used to compare means values between two groups.

3. Results

3.1. Formalin test

In the test group, formalin injection into the plantar surface of the right hind paw led to a two-phase pain response. In the early phase, the rats had a mean ± S.E.M. nociceptive score of 2.43 ± 0.05 ($n=6$), which then decreased to 1.01 ± 0.01. In the late phase, the score increased again to more than 2 (Fig. 1). These changes were significantly ($F(11,96)=30.224$, $P<0.0001$) different from those observed in the control group. In the control group, after injection of saline, no change could be detected.

3.2. Microdialysis

3.2.1. Noradrenaline

The basal level of noradrenaline in the locus coeruleus of the control group that had received injection of normal saline into the plantar surface of the right hind paw was 46.58 ± 2.03 (mean ± S.E.M.) fmol/sample ($n=6$). After injection of saline, the level increased to 77.74 ± 6.37 and then rapidly returned to the baseline level (49.84 ± 2.08). These changes were significantly different ($F(9,36)=9.79$, $P<0.0005$).

The basal level of noradrenaline in the test group (injection of 50 μl of 2.5% formalin) was 48.91 ± 0.66 fmol/sample ($n=6$). It was not significantly different from that of the control group. In the early phase of the formalin test (the first sample after injection), the concentration of noradrenaline increased to 220.5 ± 8.84 fmol/sample and then decreased to the basal level (49.66 ± 1.92) in the late phase. This increase was significantly different from the basal level ($F(9,45)=244.17$, $P<0.0005$) and significantly

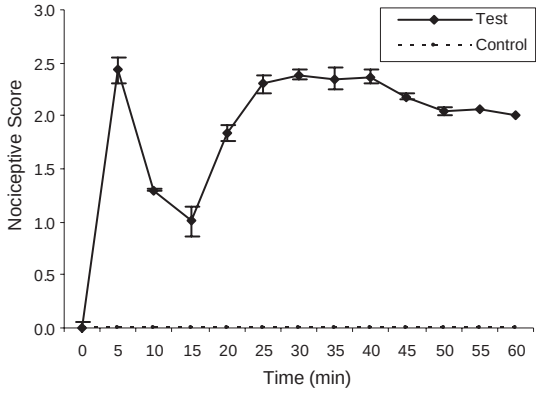

Fig. 1. The nociceptive score in test and control groups. The test group shows two phases of nociceptive behavior but in the control group does not.

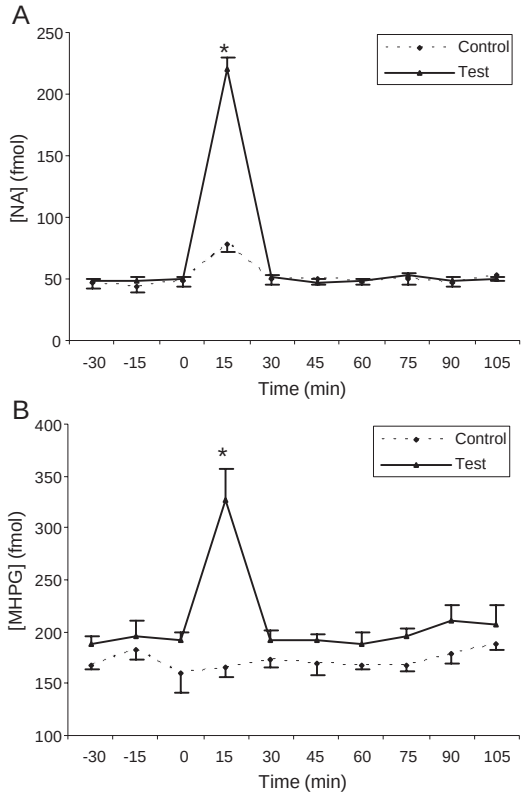

Fig. 2. (A) Mean ± S.E.M. concentration of noradrenaline in test and control groups. Time 0 corresponds to the injection time. (B) Mean ± S.E.M. concentration of MHPG in test and control groups. Time 0 corresponds to the injection time.

different from that measured in the same period in the control group ($F(1.1,11.02)=20.59$, $P<0.001$) (Fig. 2A).

3.2.2. MHPG

The basal level of MHPG in the control group was 169.77 ± 11.23 fmol/sample ($n=6$) and did not change significantly during the experiments. In the test group, the basal level was not significantly different from that of the control group (191.4 ± 4.21). In the early phase of the formalin test, the MHPG concentration increased to 327 ± 30.23 fmol/sample but then returned to the basal level in the late phase (197.6 ± 8.47). This increase was statistically significant compared with the basal level ($F(9,36)=14.91$, $P<0.0005$) and paralleled the change in noradrenaline concentration. It was significantly different from the level measured in the same period in the control group ($F(6,47)=5.45$, $P<0.004$) (Fig. 2B).

4. Discussion

We found that after the induction of pain, the noradrenaline concentration in the locus coeruleus increased almost four times its basal value (Fig. 2A). This increase happened during the early phase of the formalin test but not during the late phase. We also observed an increase in the noradrenaline concentration in the locus coeruleus in the control group at the time of saline injection. This increase, though significantly different from the basal value, can be attributed to the injection stress. The increase was much smaller than that observed after formalin injection (Fig. 2A). Therefore, part of the increase in noradrenaline concentration in the locus coeruleus is certainly due to the pain induced by formalin.

A-δ and C-fibers of peripheral nerves conduct impulses to the lumbosacral cord and to the paragigantocellularis in the rostro-ventral medulla. Direct or indirect stimulation of the paragigantocellularis enhances the activity of the excitatory amino acid (EAA) pathway, which results in glutamate and aspartate release in the locus coeruleus (Ennis et al., 1992; Singewald and Philippu, 1998). This, in turn, stimulates the noradrenergic neurons within the nucleus and enhances noradrenaline release in the locus coeruleus. The increased noradrenaline concentration in the locus coeruleus causes increased noradrenaline release in nerve terminals of the descending noradrenergic pathway in the spinal cord, leading to antinociception via stimulation of α_2-adrenoceptors (Singewald et al., 1995; Singewald and Philippu, 1998). The elevation of noradrenaline in the early phase of the formalin test, observed in our study, can cause analgesic effects mediated by α_2-adrenoceptors in the dorsal horn of the spinal cord via the descending pain control pathway (Willis and Westlund, 1997; Millan, 2002). Noradrenaline and α_2-adrenoceptor agonist applied to the spinal cord inhibit the response of dorsal horn neurons (Millan, 2002).

It is generally accepted that noradrenergic neurons of the locus coeruleus respond strongly to certain types of stress (Kawahara et al., 2000). Acute stimuli leading to increased firing of locus coeruleus neurons include noxious stimuli (Cedarbaum and Aghajanian, 1978), and noxious stimuli increase the release of noradrenaline in the locus coeruleus (Singewald et al., 1999).

The difference between noradrenaline concentrations in the two phases of the behavioral response might be due to several reasons. The first phase is believed to be produced by direct activation of nociceptive neurons by formalin, whereas in the second phase pain is generated by acute tissue injury (Hunskaar and Hole, 1987). These two different mechanisms of pain production might explain the different responses of the locus coeruleus observed in our study.

The change in MHPG concentration resembled that of noradrenaline in the test group (Fig. 2B). Hypertensive stimuli have been shown to modify the release of noradrenaline and monoamine metabolites in the locus coeruleus (Bhaskaran and Freed, 1988). Since MHPG is the main metabolite of noradrenaline, the increase in its concentration in the locus coeruleus might be a reflection of the activity of catechol-*O*-methyltransferase enzyme activity which metabolizes noradrenaline in the nucleus (Morier-Teissier and Rips, 1987).

This study underlines the role of the locus coeruleus in the control of chemical and tonic pain, and also shows that in the early phase of the formalin test, the pain induced can be attributed to the direct irritating effect of formalin on the pain nerve terminals (A-δ and C-fibers) without inflammation of the injection site. The increase in locus coeruleus activity caused by pain in the early phase of formalin test seems to be attributed to the direct stimulation of nerve fibers present at the injection site. In the late phase of the test, inflammation of tissues induces signs of nociception; however, it seems that this nociceptive state does not affect the locus coeruleus.

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