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Exposure to Stress Alters the Effects of Dynorphins in the Hot Plate Test

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Summary

The analgesic effects of intracerebroventricularly (i.c.v.) administered dynorphin A(1-13) and its analog dynorphin A(1-10)amide using the hot plate test were studied in mice. Both dynorphins applied i.c.v. by the freehand method had an analgesic effect but no effect was seen when applied i.c.v. through an implanted cannula. Moreover, freehand i.c.v. injection of saline increased the time of immobility in the forced swimming test and glycaemia levels compared with intact mice. In contrast to the freehand injection, saline administration through an implanted cannula did not influence the immobility of animals in forced swimming test when compared with the intact controls. These results suggest that 1) the freehand method is very stressful procedure of administration which could influence the effects of dynorphins in the hot plate test and 2) dynorphins exert an analgesic effect in the hot plate test only when combined with a stressor (freehand i.c.v. injection).

Key words
Stress – Analgesia – Dynorphin – Hot-plate

Introduction

Various stressors produce opioid or non opioid-mediated analgesia depending on the severity of stress, its behavioural significance, etc. (Miczek *et al.* 1982, Lewis *et al.* 1980, Terman *et al.* 1986, Sadowski and Panocka 1993). Stress-induced analgesia can be influenced by many drugs and also drug-induced antinociception can be modified by different stressors. Analgesia produced by continuous cold-water swimming (non-opiate analgesia) was altered by pretreatment with morphine (Grisel *et al.* 1993). Short cold-water swimming which did not result in analgesia in mice, shifts the dose-response curve for morphine analgesia to the left (Vanderah *et al.* 1993). Restraint – another frequently used stressor – potentiates the magnitude and duration of analgesia following administration of several opioid agonist (morphine, enkephalin analog DAGO) as compared to non-stressed controls (Calcagnetti *et al.* 1992, Levesque and Holtzman 1993, Woolfolk and Holtzman 1993).

Freehand injection is routinely used for the central administration of drugs in mice by many researchers. However, already Boschi *et al.* (1981) pointed out that this method could be extremely stressful to non-anaesthetized animals which may affect the results.

In previous experiments (Starec *et al.* 1996), we have shown that the analgesic activity of dynorphins (drugs known to have unique binding characteristics among the kappa-opioids) play a physiological role as endogenous pain modulator during stress conditions. We found that the intracerebroventricularly (i.c.v.) freehand administered dynorphin A(1-13) and its analog dynorphin A(1-10)amide exerted revealed analgesic activity under stress conditions (swimming, horizontal vibration) when measured by the tail-flick method, although they did not show any antinociceptive

effects by themselves. But we also noticed longer tail-flick latencies even in saline controls after freehand i.c.v. administration compared with intact mice. Therefore, in the present experiments, we decided to study the analgesic effects of freehand i.c.v. administration of dynorphin A(1-13) and its analog dynorphin A(1-10)amide using another analgesic test – the hot plate method. We also studied the influence of i.c.v. administration of the vehicle (saline) using the freehand method on blood glycaemia levels (which is a good stress marker) and on the immobility time in the Porsolt's swimming test. In the second series of experiments, we compared the effect of freehand i.c.v. administration with i.c.v. administration using an implanted intracerebral cannula (presumably a less stressful route of administration).

Materials and Methods

Animals

Male, white IRC mice weighing between 25 to 30 g were used in all experiments. The mice were kept in cages of 10 animals each, which had free access to food and water. They were housed under natural day/night conditions for at least two weeks before the experiments.

Analgesic assessment

Analgesic activity was measured using the hot plate method according to Eddy and Leimbach (1953). Mice were placed on a hot plate maintained at 55 °C. The times for licking or jumping were recorded. Cutoff time was 30 s.

Drugs

The following drugs were used: dynorphin A(1-13) in the dose of 25 nmol/mouse and dynorphin A(1-10)amide in the dose of 60 nmol/mouse. Both dynorphins were obtained through the courtesy of Tsumura INC, Japan.

Injections

1. Freehand i.c.v. administration

Compounds or vehicle were delivered into the lateral cerebral ventricle using a modification of the method of Haley and McCormick (1957). The mice were lightly anaesthetized with ether, an incision was made in the scalp and the bregma was located. Compounds or saline were injected in awake, hand-held mice directly through the skull at a point 2 mm caudal and 2 mm lateral to the bregma at a depth of 3 mm using a 10 microlitre Hamilton syringe. All i.c.v. injections were made in a volume of 3 microlitres. Special experiments were made to verified the correct i.c.v. application by injecting methylene blue at the same speed and volume as for drug administration. After sacrifice, the brains were removed, sectioned frontally and examined microscopically for the presence of dye particles in the ventricles. About 85 % of mice were correctly injected. Naloxon was injected subcutaneously 15 min before i.c.v. application of the drugs.

2. Implanted chronic cannula

In other groups of mice, intracerebral cannulae were implanted as described by Boschi *et al.* (1981). Intramuscular needles were cut perpendicularly to the axis into 13 mm long segments to serve as guiding cannulae. The animals were anaesthetized with pentobarbital and their skulls were shaved. The scalp was incised longitudinally and retracted from the midline. A hole was made with a dental drill (burr size 1 mm) at the level of the left lateral ventricle. The cannula (0.8 mm of external diameter) was cemented in place with dental cement. Mice were allowed to recover one week before the experiments.

Porsolt's forced swimming test

The used forced swimming test was a modification of that described by Porsolt *et al.* (1978). Mice were forced to swim for 6 min inside a vertical cylinder of transparent plastic with a diameter 40 cm and 60 cm high filled with water 32±1 °C to 30 cm depth. The animals could not escape. The total duration of immobility during the last 3 min was recorded. A mouse was judged to be immobile whenever it remained floating in the water in an upright position, making only very small movements necessary to keep its head above water. The behavioural assessment during the test swim was made by an observer unaware to which group the mice belonged.

Glycaemia

Mice were sacrificed, blood samples were withdrawn, centrifuged and serum glycaemia was determined using the ortho-toluidin method.

Data analysis

Statistical significance of the data was determined by an analysis of variance (Anova). Means and standard deviation (S.D.) are given. In all cases, $p < 0.05$ was considered statistically significant.

The experiments were approved by the ethical committee of the Third Medical Faculty, Charles University, Prague.

Results

Hot plate test

We found significantly higher response latencies in the hot plate test in mice treated with dynorphin A(1-13) and its analog dynorphin A(1-10)amide 15 min after i.c.v. freehand administration compared with the vehicle controls. Also i.c.v. administration of saline had slight (but not

significant) analgesic effect when compared with intact non-injected animals (Fig. 1).

Porsolt's forced swimming test and glycaemia levels

The direct freehand i.c.v. injection of saline increased the time of immobility in the 4th, 5th and 6th minute of the forced swimming test compared with intact mice (Fig. 2). Also glycaemia levels significantly increased in animals injected freehand i.c.v. with saline 15 min after its administration when compared with non-injected animals (Fig. 2).

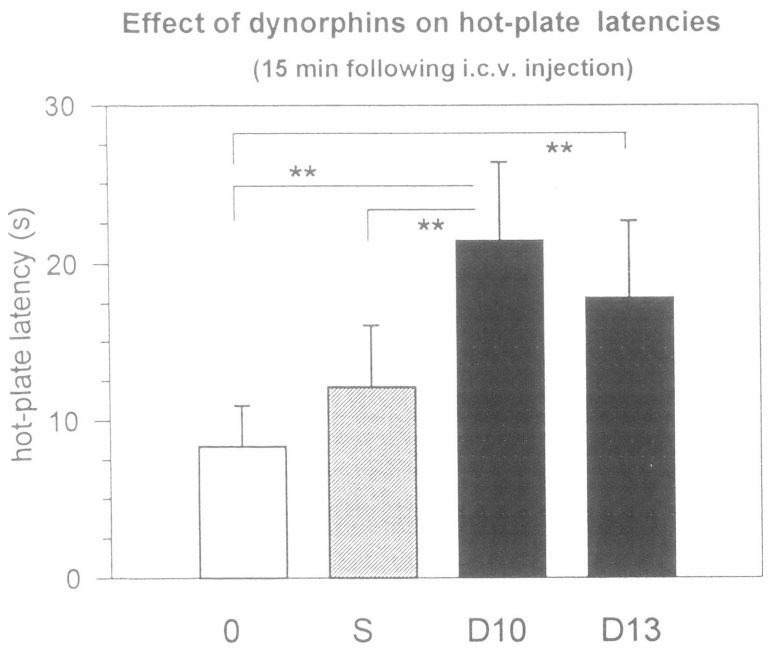

Fig. 1. *Effect of dynorphin A(1-13) and dynorphin A(1-10)amide 15 min after their intracerebroventricular applications using freehand method on hot plate test. 0 = intact controls, S = saline freehand i.c.v. controls, D10 = dynorphin A(1-10)amide freehand i.c.v., D13 = dynorphin A(1-13) freehand i.c.v., ** $p<0.01$. The results are means of hot plate latencies ± S.D. $n=20$ mice.*

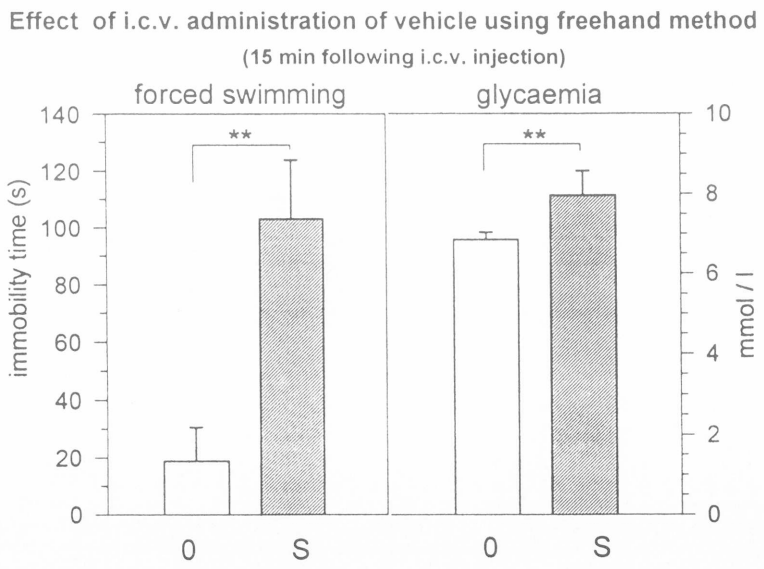

Fig. 2. *Effect vehicle (saline) 15 min after intracerebroventricular administration using freehand method on the time of immobility in Porsolt's forced swimming test and glycaemia. 0 = intact controls, S = saline freehand i.c.v. controls. Shown are mean times of immobility and mean glycaemia levels ± S.D., $n=15$ in all groups, ** $p<0.01$.*

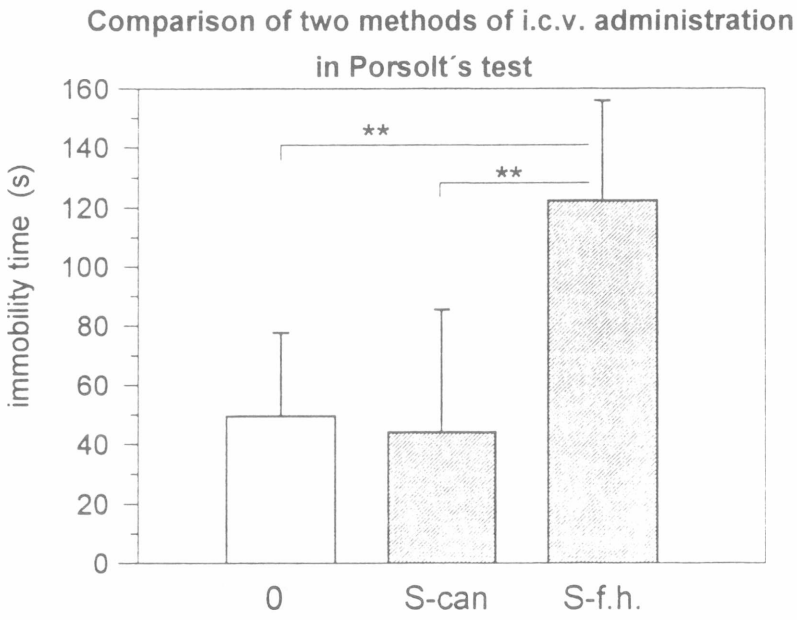

Fig. 3. *Comparison of two methods of i.c.v. administration on the time of immobility in Porsolt's forced swimming test (freehand method compared with injection using implanted cannula). 0 = intact controls, S-can = saline i.c.v. through implanted cannula, S-f.h. = saline freehand i.c.v., ** $p<0.01$. The results are mean times of immobility ± S.D., $n=15$ in all groups.*

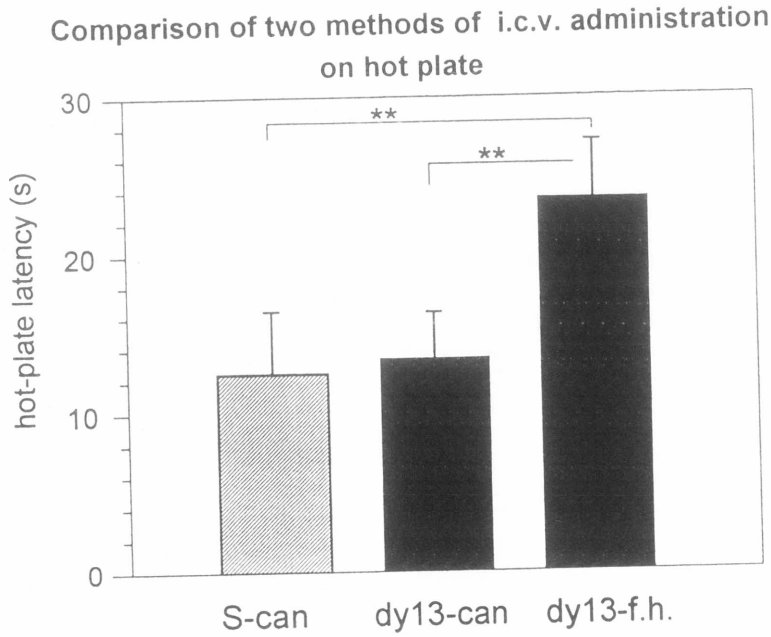

Fig. 4. *Comparison of two methods of i.c.v. administration of dynorphin A(1-13) on the hot plate test (freehand method compared with injection using implanted cannula). S-can = saline i.c.v. through implanted cannula, 13-can = dynorphin A(1-13) i.c.v. through implanted cannula, dy 13-f.h. = dynorphin A(1-13) freehand i.c.v,. ** $p<0.01$. Results are mean plate latencies ± S.D., $n=15$ in all groups.*

Comparison of free hand/cannula i.c.v. administration in forced swimming test

In contrast to the freehand injection, saline administration through the implanted cannula did not influence the immobility of animals in the 4th, 5th and 6th min of forced swimming test when compared with the intact controls. (Fig 3).

Comparison of free hand/cannula i.c.v. administration on the hot plate test

There was no statistically significant difference between the effect of saline and dynorphin (1-13) when administered i.c.v. using an implanted cannula, but again dynorphin (1-13) significantly increased hot plate latencies in mice injected by the i.c.v. freehand method (Fig. 4).

Discussion

It is generally accepted that drug-induced antinociception can be modified by different stressors. The most important finding of this study is that dynorphin A(1-13) and dynorphin A(1-10)amide revealed analgesic activity measured by the hot plate method only when administered i.c.v. using the free hand technique, but showed no analgesic effect after i.c.v. application using an implanted cannula. Thus, our results confirmed the statement of Boschi *et al.* (1981) that the freehand injection (which is routinely used for intracerebroventricular administration of drugs in mice) could be stressful to animals and may affect the experimental results.

Furthermore, it was found in the present study that freehand i.c.v. administration of only vehicle (saline) caused impairment of motility in Porsolt—s test and an increase in blood glucose levels. This again supports the presumption that this technique of administration is a stressor which may affect our hot plate results.

In the literature, there are conflicting data concerning the analgesic activity induced by centrally (i.c.v) administered dynorphin A(1-13). By itself, it produces analgesia in the tail pinch test (Herman *et al.* 1980, Kaneko *et al.* 1983, Nakazawa *et al.* 1985) hind paw pressure test (Hayes *et al.* 1983, Kishioka *et al.* 1992), acetic acid induced writhing test (Nakazawa *et al.* 1985, Gairin *et al.* 1988, Kishioka *et al.* 1992) and cold water tail-flick test (Tiseo *et al.* 1988, 1990), but not in the radiant tail-flick test (Friedman *et al.* 1981, Pentel *et al.* 1995).

Conflicting are also data concerning analgesic activity evaluated by the hot plate method. In rats, Hayes *et al.* (1983) found that dynorphin A(1-13) injected i.c.v. using a permanent cannula was ineffective in the hot plate test (52 °C) even at a dose of 80 μg/rat. In contrast, Tilson *et al.* (1986) reported increased latencies in hot plate that (55 °C) in rats receiving i.c.v. 15 μg of dynorphin A(1-13). The differences between these two experiments concerned the strains of rats, temperature of the plate surface, but also the fact that animals in Tilson's experiments were tested for motor activity and for startle reactivity to an acoustic stimulus before the hot plate test. Thus, the observed increased dynorphin analgesic activity could be caused by stressful manipulation prior to the nociceptive stimulus. In mice, Kostrzewa *et al.* (1992) showed that animals treated i.c.v. (using the freehand method) with dynorphin (1-13) in the dose 20 μg/mouse had prolonged hot plate latencies similar to our results. Moreover, mice were prestressed by three preinjections before the hot plate test. We found that the same dose of dynorphin given i.c.v. through an implanted cannula (which is supposed to be a less stressful manipulation) instead of the freehand method had no or very weak analgesic potency in the hot plate test.

In a previous paper (Starec *et al.* 1996), we confirmed the finding of Nakazawa *et al.* (1985) that i.c.v. injection (even using the freehand method) cause no analgesia in the radiant heat tail-flick test. The reason why stressful administration potentiated the effect of dynorphin A(1-13) in the hot plate test (and not in tail-flick) could be explained by different reflex pathways involved in both tests. It is generally accepted that the type of pain test used is of the utmost importance because different pain tests yield different results even when the same pharmacological manipulation is used (Suh *et al.* 1994, Nakazawa *et al.* 1985). Responses in the tail-flick test can be governed by a neuronal loop at the spinal level (Amit and Galina 1986). On the other hand, the hot plate test is a procedure thought to involve an integrated escape response that may be mediated at central nervous system levels higher than the spinal cord (Eddy and Leimbach 1953, Amit and Galina 1986). Moreover, besides nociception, several other processes, including alteration in attention, motivation and general perception may be involved in the performance deficits.

In conclusion, all these findings suggest that 1) dynorphins exhibited their analgesic effect in the hot plate test in mice only when administered i.c.v. by freehand injection and 2) freehand i.c.v. injection appears to be a highly stressful procedure of administration which could influence the effects of dynorphins in the hot plate test. It therefore seems to be advisable to use a less stressful method for i.c.v. administration in some experiments. The method of the i.c.v. administration should be specified in details in reports on this kind of research.

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Reprint requests

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