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BRIEF COMMUNICATION

Chronic Intrathecal Cannulation Affects Hypothalamic Opioids Depending on the Technique Employed

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DIB, B., P. SACERDOTE AND A. E. PANERAI. *Chronic intrathecal cannulation affects hypothalamic opioids depending on the technique employed.* PHARMACOL BIOCHEM BEHAV **40**(2) 449–451, 1991.—Previous studies have shown that chronic intrathecal cannulation can interfere with the homeostasis of central opioid peptides. These results show that beta-endorphin and Met-enkephalin concentrations did not change in the hypothalamus of rats bearing a chronic cannula inserted between C_8–T_1 up to L_3 and fixed to the processus transversus T_1. These results suggest that chronically cannulated rats can be considered as normal when studying hypothalamic beta-endorphin or Met-enkephalin concentrations, and used in physiological studies, depending on the technique employed.

Beta-endorphin Met-enkephalin Chronic cannulation Intrathecal Intraperitoneal

SEVERAL techniques for intrathecal cannulation have been employed, the most frequently used being the one described by Yaksh and Rudy (8). According to this method a small polyethylene cannula is inserted through a puncture in the atlanto-occipital membrane and secured to the skull. Following this surgical procedure, however, paralysis of the hind legs due to damage of the spinal cord often results. In addition, a recent study has demonstrated that with no motor deficit rats cannulated with this classical technique show changes in hypothalamic beta-endorphin and Met-enkephalin concentrations similar to those observed in animals bearing a transection of peripheral nerves (6,7), or a complete spinal transection (6). It has been suggested that results obtained from rats cannulated with the classical technique can be biased and could not be used for physiological or pharmacological studies, at least as far as neuropeptides were concerned.

In the search for a better cannulation technique, in order to allow physiological and pharmacological studies, we evaluated the hypothalamic beta-endorphin and Met-enkephalin concentrations in rats cannulated according to another method previously reported (1); rats cannulated according to this technique seem to be normal as far as hypothalamic neuropeptides are concerned.

METHOD

Adult male Sprague-Dawley CD rats (Charles River, Calco, Italy) weighing between 250–280 g were housed at 22±2°C with a light-dark cycle of 14–10 hours and free access to water and dry pellets were used in all experiments. The animals were divided into five groups of ten. Group one (control) did not receive any treatment; group two was cannulated according to Yaksh and Rudy (8); group three was cannulated with an alternative method (1); group four had a cannula inserted in the intraperitoneal space in order to control for a nonspecific irritative focus; group five underwent all experimental procedures of group three, in this case the cannula was inserted intrathecally and immediately removed (sham group).

Both cannulation techniques were applied as previously described. The main difference between the two techniques is the different site insertion and fixation of the cannula to the rat. In the classical method, in fact, the cannula was inserted through a puncture in the atlanto-occipital membrane and fixed to the skull, while in the other method we tested, the cannula was inserted at C_8–T_1 and is fixed directly on the processus transversus of T_1. In both cannulations the tip was pushed as far as L_3.

On day eight after surgery, all rats were killed the same day

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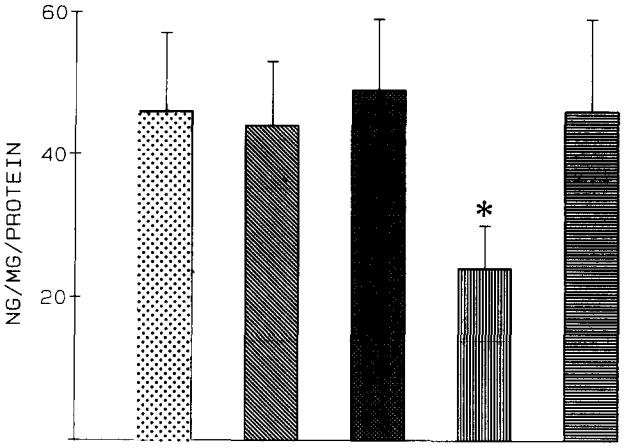

FIG. 1. Beta-endorphin concentrations in the hypothalamus of control (dotted bars) and sham-operated (hatched bars) rats; rats bearing an intraperitoneal cannula (cross-hatched bars), operated by the method of Yaksh (vertical line bars) and operated by the alternative method (horizontal line bars) (see text for details). Ten rats were used in each situation mentioned above. *$p<0.05$ vs. all other experimental groups.

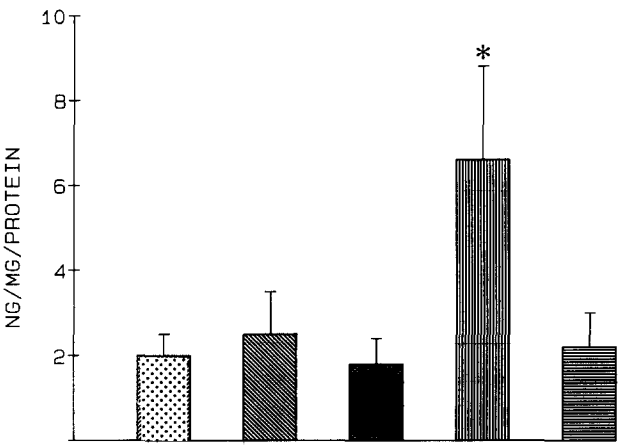

FIG. 2. Met-enkephalin concentrations in the hypothalamus of control (dotted bars) and sham-operated (hatched bars) rats; rats bearing an intraperitoneal cannula (cross-hatched bars), operated by the method of Yaksh (vertical line bars) and operated by the alternative method (horizontal line bars) (see text for details). See the legend of Fig. 1 for the number of rats. *$p<0.05$ vs. all other experimental groups.

by microwave irradiation (4), the hypothalamus removed according to a procedure described by Glowinski and Iversen (2), homogenized in 0.1 N acetic acid, centrifuged and pellet and supernatants separated and frozen till assay for protein or peptides determination. Proteins were measured according to Lowry et al. (3), and peptides by radioimmunoassays previously described in detail (4,5). In brief, beta-endorphin and Met-enkephalin were measured by radioimmunoassay with C-terminal-directed antibodies specific for beta-endorphin or Met-enkephalin. The beta-endorphin antiserum was obtained against synthetic human BE, and is directed toward the C-terminal of the peptide as it is also demonstrated by the 100% cross-reactivity with human beta-lipotropin and the low cross-reactivity with camel BE (5%). Despite the cross-reactivity with beta-lipotropin, a minor cross-reactivity was also observed toward equimolar Met-enkephalin (0.1%), but not with Leu-enkephalin, dynorphin, alpha-MSH, beta-MSH, substance P, somatostatin, TRH, corticotropin releasing hormone, neurotensin, vasopressin, bombesin, cholecystokinin, vasoactive intestinal peptide, insulin, FSH, LH, prolactin, growth hormone, morphine, naloxone, or the cytokines interleukin-1 alpha or beta, and tumor necrosis factor. The antiserum for Met-enkephalin was raised in the rabbit against the synthetic peptide. The antiserum shows 0.5% cross-reactivity with Leu-enkephalin. No cross-reactivity was observed with the same peptides, hormones, cytokines and drugs tested with the beta-endorphin antiserum. All radioimmunoassays were performed in one run for each peptide, in order to minimize assay-related variations. The analysis of variance, followed by the Dunnett's test for multiple comparisons was used for the statistical analysis of data.

RESULTS

Figure 1 shows that hypothalamic beta-endorphin concentrations are comparable in the control and sham-operated rats, in animals bearing a cannula in the intraperitoneal space, and in rats that had a cannula inserted according to the second method, while they were significantly decreased in rats that had the cannula inserted according to the classical method ($p<0.05$). Figure 2 shows the concentrations of Met-enkephalin in the hypothalamus of the same rats. As it clearly appears, the concentrations are significantly higher in the animals in which the cannula was inserted according to the method of Yaksh et al. (8), than in all other experimental groups ($p<0.05$).

DISCUSSION

The data presented show that differences in the method used for inserting a cannula into the intrathecal space of the rat induce different effects on basal values of hypothalamic beta-endorphin and Met-enkephalin. This observation is important in view of the use of cannulated animals for physiological or pharmacological studies, especially those dealing with neuropeptides. The main difference between the two methods seems to lie in the different sites of insertion and fixation of the cannula. In the classical method, the insertion and fixation were made through a puncture in the atlanto-occipital membrane and secured to the skull. This insertion and fixation allows for intrathecal movements of the tip of the cannula that follow movements of the head of the animal, with the consequence of continuous irritation and possible lesions. In the second method, the cannula is inserted between C_8–T_1 and fixed to T_1 and, therefore, it does not move with the head, and intrathecal movements are almost totally prevented [for more details, see (1)].

In conclusion, the second method has to be preferred when one needs a reliable experimental model for physiological or pharmacological studies involving neuropeptides, e.g., the study of pain thresholds or of analgesic drugs.

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