EFFECT OF COLCHICINE ON ANALGESIC EFFECTS OF MORPHINE AND DADL IN RATS

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The microtubules are receiving ever-increasing attention on the part of research workers in recent years as structures involved in the intracellular transmission of the signal in various cells [2, 4]. One of the agents preventing aggregation of microtubules is colchicine [3]. By interacting specifically with tubulin colchicine prevents the formation of microtubules and ruptures the bond with receptors located on the plasma membrane of receptor cells [1, 2]. It is not known whether tubulin is a binding component similarly for opiate receptors.

The aim of this investigation was to study the action of colchicine on the analgesic effects of morphine and DADL, which are agonists of μ- and σ-opiate receptors in the tubulin—microtubules system.

EXPERIMENTAL METHOD

Experiments were carried out on 20 conscious male rats weighing 250-300 g. Under general anesthesia (hexobarbital) the animals were scalped and guiding cannulas were inserted into the lateral ventricle. The rats were placed 1-3 days after the operation in a transparent plastic chamber, restricting the animal's movements, and with the tail exteriorized.

The latent period (LP) of the tail withdrawal behavioral response (TWR) to a nociceptive temperature stimulus (a focused beam of light, directed on the animal's tail) was recorded automatically by means of an F-5080 frequency-chronometer. Stimulation of this kind caused a burn of the tissues of the tail after 50 sec. The preparations used, namely morphine (50 μg), D-Ala-D-Leu-enkephalin (50 μg), from "Serva," and colchicine, in doses of 1, 10, and 20 μg (from "Merck") were injected into the lateral ventricle in a volume of 5 μl of 0.9% NaCl.

The animals were divided into four groups with five rats in each group. Group 1 received colchicine in a dose of 20 μg, group 2 — 10 μg, group 3 — 10 μg, and group 4 — 1 μg colchicine. Rats of groups 1, 2, and 4 also received an injection of morphine after colchicine, in a dose of 50 μg, and rats of group 3 received DADL in a dose of 50 μg.

EXPERIMENTAL RESULTS

The experiments showed that in the experimental animals of groups 1 and 2 the average LP of TWR was 13.6 ± 1.08 sec. After injection of morphine (50 μg) an analgesic effect was observed, as shown by absence of TWR for 50 sec. After a further 50 sec, in order to avoid a burn, the temperature stimulation was discontinued. Restoration of TWR was observed 2.5 ± 0.2 h after the injection of morphine, with LP of 15 ± 2.5 sec, not significantly different from LP of TWR before injection of morphine. One day later these same animals were given colchicine: group 1 — 20 μg, group 2 — 10 μg. Next day the background values of LP of TWR of the rats of groups 1 and 2 were 9.1 ± 0.7 and 15.9 ± 0.7 sec. After injection of morphine (50 μg) LP of TWR showed no significant change (10.1 ± 0.7 and 17.1 ± 1.0 sec), i.e., the analgesic effect was absent. The effect of blockade of the analgesic action of morphine in the animals of group 2 lasted 5 ± 1 sec (Fig. 1).

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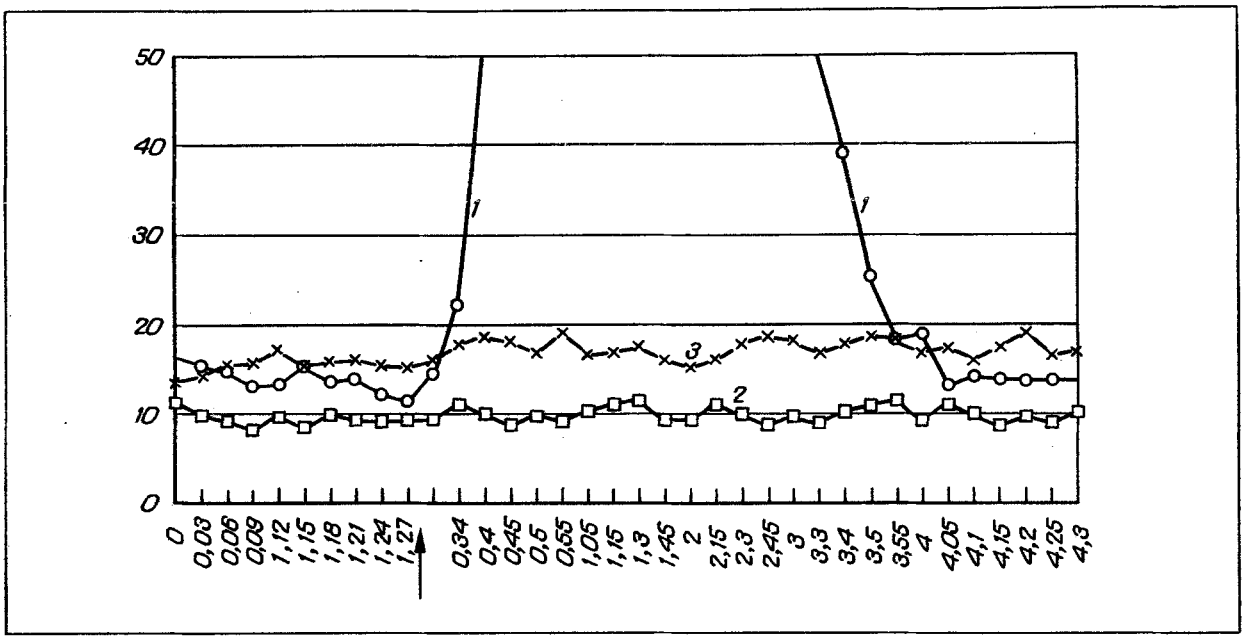

Fig. 1. Analgesic effect of morphine (50 μg) when injected into the lateral ventricle of control animals (n = 5) (1) and its absence after injection of colchicine in a dose of 20 μg (2) and 10 μg (3). Here and in Fig. 2: abscissa, LP of TMR; ordinate, time (min, h). Arrows indicate injection of morphine.

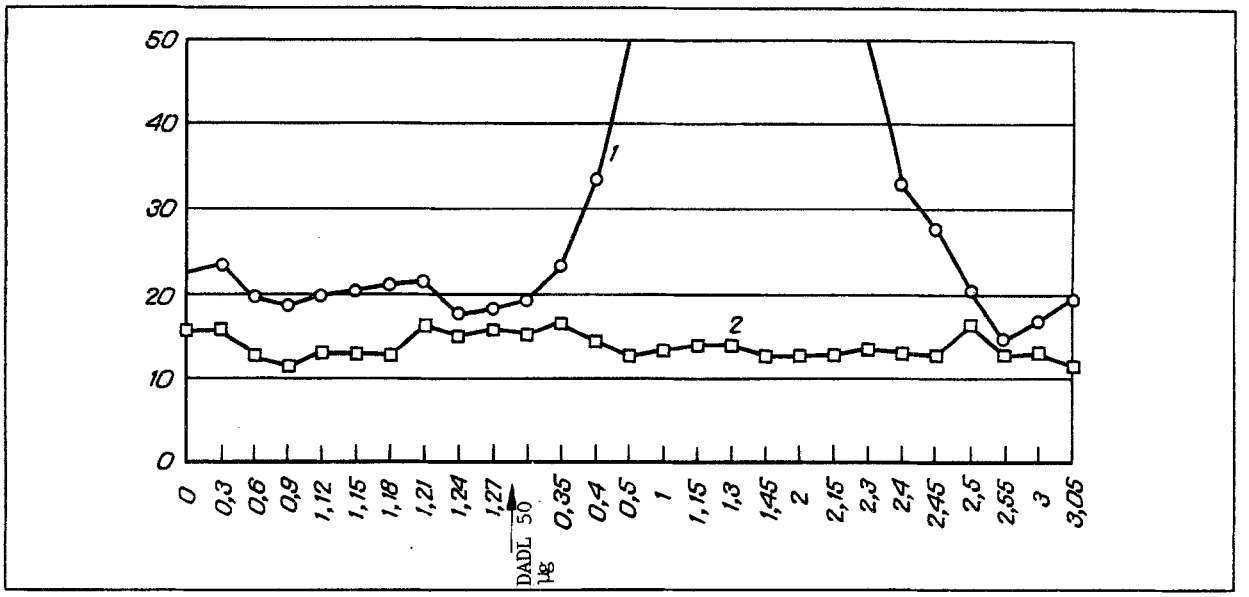

Fig. 2. Analgesic effect of DADL (50 μg) when injected into lateral ventricle of animals of control group (n = 5) (1) and its absence after injection of colchicine in a dose of 10 μg (2).

In the animals of group 3 LP of TWR before injection of DADL was 19.8 ± 2 sec. After injection of DADL (50 μg) an analgesic effect was observed, namely disappearance of TWR for 50 sec during the action of the stimulus, and in order to avoid burning, the stimulus was discontinued. TWR was restored 1.5 ± 0.1 h later, when its LP was 19.9 ± 1.9 sec. After 1 day the same animals were given an injection of colchicine (10 μg). Next day, LP of TWR was 13.1 ± 0.7 sec, and after injection of the same dose of DADL it did not change significantly (13.1 ± 0.5 sec), i.e., no analgesic effect was present (Fig. 2).

In the rats of group 4 injection of colchicine (1 μg) did not significantly change LP of TWR (13.6 ± 1.1 and 12.9 ± 0.5 sec, respectively). Morphine (50 μg) preserved its analgesic effect for 2.5 ± 0.2 h, after which LP of TWR was restored to 13.3 ± 0.7 sec.

The experiments thus showed that colchicine in doses of 10 and 20 μg blocks the analgesic effects of morphine and DADL in a dose of 50 μg. However, a dose of colchicine 1 μg has no such action. The results raise the question of whether the observed effects of blocking of opiate receptors are the result of disaggregation of tubulin or whether colchicine causes destruction of neurons of structures adjacent to the periaqueductal system of the vein. There is evidence in the literature that intradental injection of colchicine in a dose of 2.5 μg leads to destruction of the dental granule cells of the hippocampus [5]. Colchicine in a dose of 3.5 μg intradentally caused reversible anatomical changes in the hippocampal pyramidal cells, and destroyed them in a dose of 25 μg. Direct injections of colchicine into certain regions of the CNS cause local death of cells, for example, colchicine destroys granule cells and Purkinje cells in the cerebellum [5], as well as neurons in the entorrhinal cortex [9] and striatum [8]. However, the same workers observed the absence of any destructive effect of colchicine in a dose of 2.5 μg on microinjection into the neocortex.

In the present experiments, after injection of colchicine into the cerebral ventricle of rats its concentration in the CSF reached 10 μM, for the volume of the cerebral ventricle in rats is 100 μl [7]. According to data in the literature, colchicine in micromolar concentrations has no toxic action, and is specific for tubulin disaggregation [1]; consequently, in the present experiments its action was specific. In view of the specificity of action of colchicine it can be postulated that tubulin is an intracellular transmitter of the analgesic effects of morphine and DADL. Cytostatics of colchicine type can be used as long-term blockers of narcotic analgesics and opioid peptides.

LITERATURE CITED

1. A. I. Esakov, Dokl. Akad. Nauk SSSR, **250**, No. 5, 1274 (1980).
2. J. Atema, Microtubules and Microtubule Inhibitors, M. Borgers and M. de Brabander (eds.), Amsterdam (1975), pp. 247-257.
3. P. Dustin, Microtubules, Berling (1978).
4. S. R. Hameroff and R. C. Watt, J. Theor. Biol., **98**, No. 4, 549 (1982).
5. R. B. Goldschmidt and O. Steward, Proc. Nat. Acad. Sci. USA, **77**, 3047 (1980).
6. R. B. Goldschmidt and O. Steward, Neuroscience, **7**, 695 (1982).
7. A. Mitro and M. Palkovits, Morphology of Rat Brain Ventricles, Ependyma and Periventricular Structures, Basel (1981), pp. 40-43.
8. B. Siegfried, J. Fischer, and J. Bures, Neuroscience, **5**, 529 (1980).
9. O. Steward and R. B. Goldschmidt, Life Sci., **35**, 43 (1983).