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Antinociceptive Effect of Intrathecally Administered Serotonin

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It has been suggested that serotonin neurons and their pathways may mediate sensitivity to nociceptive stimuli by activating the descending inhibitory mechanisms at the spinal cord. This antinociceptive effect may be induced by direct administration of serotonin into the cerebrospinal fluid pathways. The experiment is designed to demonstrate the changes in the tail-flick response latency after the intrathecal injection of serotonin. Serotonin, 100 or 200 μg, administered into the lumbar intrathecal space, produced an analgesic effect for as long as 40 minutes. Behavioral and morphologic observations after serotonin injections showed no adverse reaction. It is assumed that serotonin molecules penetrate the spinal cord tissue and activate the antinociceptive serotonergic pathways. (Key words: Analgesia; Serotonin; Spinal cord, subarachnoid space, tail flick.)

Serotonin is a putative central nervous system neurotransmitter. Serotonin-containing neurons in raphe nuclei send ascending and descending fibers to modulate brain functions. Descending fibers project to gray matter at all levels of the spinal cord.[1,2] Both microinjection of morphine[3–5] and electrical stimulation[6,7] in the raphe nuclei produce analgesia. This analgesic effect can be antagonized by depletion of serotonin with p-chlorophenylalanine, an inhibitor of serotonin synthesis,[8,9] or by high spinal-cord transection.[10,11] These data suggest that serotonergic pathways acting as descending inhibitory mechanisms exert their antinociceptive effect by releasing serotonin in certain neuronal synapses at the spinal-cord level. The experiment to be described has been designed to demonstrate the changes in the tail-flick response latency after the intrathecal administration of serotonin.

Methods

White male Sprague-Dawley rats weighing 400 to 500 g were used for the experiment. A polyethylene catheter (PE 10) was inserted through the cisternal membrane of each anesthetized rat. The catheter was advanced to the L1–3 subarachnoid space and its position checked roentgenographically after injection of contrast medium. The other end of the catheter was connected to a hub and was fixed to the back of the skull with sutures.

After a four-day or longer recovery period, the rat was placed in a restraining rectangular box. The tail end of the box has an opening big enough for the protruding tail. Several openings at the front end allowed entrance of sufficient air for breathing. The most distal 5 cm of the tail was blackened with a felt-tipped pen, and during repetitive testing a light was randomly focused along this extent.

The intensity of radiant heat was adjusted to produce the baseline latency, ranging from 3 to 4 seconds. A baseline latency was established by four trials, with a two-minute intertrial interval. The first trial was discarded and the mean response latency (BLn) of the last three trials was calculated in seconds.

Physiologic saline or drug solution was injected through the extension catheter outside the box so that the rat was not disturbed (fig. 1). The volume of the catheter was less than 50 μl. Air of the exact volume of the dead space was injected immediately after the drug was administered, to wash the catheter.

Table 1 shows the experimental procedures of control and study groups. After administration of the drug at 15-minute intervals for 120 minutes, the mean response latency (Tn) was computed from the data of three trials at two-minute intervals. When the rat failed to respond within seven seconds, the lamp was switched off, because continuous exposure to radiant heat is injurious to the skin. The degree of analgesia (DAn) was calculated as the percentage of the maximum possible effect according to the formula[9]:

$$DAn = \frac{Tn - BLn}{7 - BLn} \times 100$$

Behavioral response to drug injections was observed carefully during and after the experiments. In two animals, seven days after the serotonin injections, the spinal cord was exposed to verify the position of the catheter tip, and gross and microscopic appearances of the cord were inspected.

Table 1. Experimental Procedures

Drug administered	Number of Experiments	Dosage and pH
None	2	—
Sterile water with preservative (benzyl alcohol 0.9 per cent)	2	50 μl, pH 6.7
Saline solution (50 μl), then serotonin (100 μg in saline solution)	2	50 μl, pH 7.3
Saline solution (50 μl), then serotonin (100 μg in saline solution), then repeat serotonin, 100 μg	1	50 μl, pH 7.3
Saline solution (50 μl), then serotonin (200 μg in saline solution)	7	50 μl, pH 7.3

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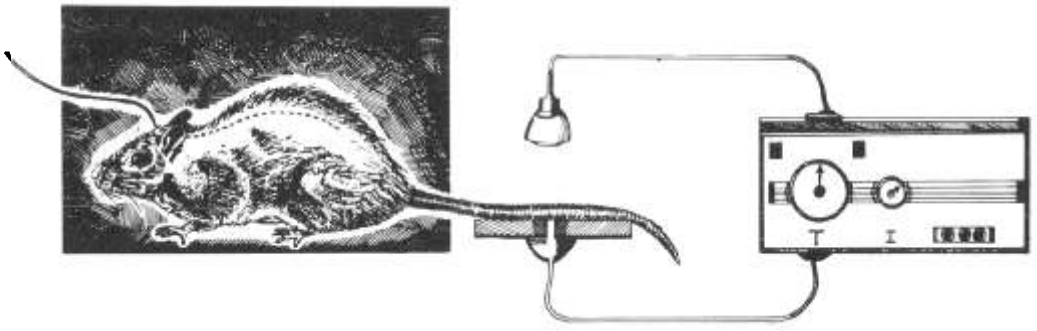

FIG. 1. Diagrammatic representation of experimental design. Drug solutions are injected outside box via a connecting catheter.

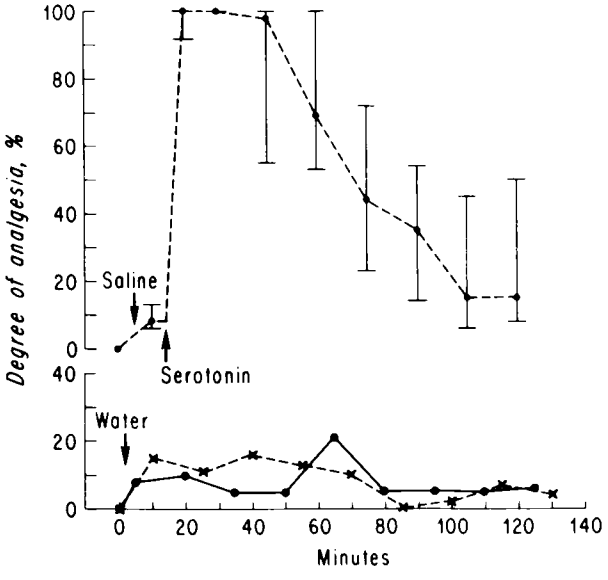

FIG. 2. Changes in degree of analgesia. Control studies in two animals with injections of water, 50 μl (*bottom*); median of six animals with highest and lowest ranges following injections of serotonin, 200 μg (*top*).

Results

Three of 17 rats failed to establish a stable baseline latency and were discarded from the study.

Injections of sterile water with preservatives (0.9 per cent benzyl alcohol) caused some withdrawal response, indicative of irritant effects. The responses to injections of drug solution were brief (less than 20 seconds), and no abnormality in behavior or motor function was detected immediately or as long as two weeks after the injections. The gross and microscopic observations of the spinal cord at the level of the catheter tip, seven days after the initial injection of serotonin (200 μg), showed no abnormal reaction.

Rats that had no injection or injections of sterile water demonstrated fluctuation in the degree of analgesia (fig. 2), the maximal change being 21 per cent. Injections of saline solution (50 μl) to assure the patency of catheters did not change DAn appreciably (less than 15 per cent). When serotonin, 100 μg, was injected, there was a rapid onset of total analgesia in two rats. In a third animal, two injections of serotonin, 100 μg, were required to establish total analgesia (fig. 3). The duration of total analgesia was less than 20 minutes.

After injections of serotonin, 200 μg, total analgesia with rapid onset was produced in six of seven rats (fig. 2). In the animal with no analgesic effect, a leak at the site connecting the extension catheter and the subarachnoid catheter was later found; results from this animal were excluded from the study. Durations of total analgesia ranged from 20 to 40 minutes. By the end of two hours the analgesic effect had virtually disappeared.

Discussion

These studies indicate that direct administration of serotonin in the lumbar subarachnoid space produces analgesia in animals that have no apparent serotonin deficiency. It is reasonable to assume that intrathecally administered serotonin molecules penetrate spinal cord tissue and inhibit nociceptive transmission, possibly by affecting the specific serotonin receptors. However, the artificial flooding of small areas of the spinal cord may not mimic the effects of the minute quantities of the transmitter that are normally re-

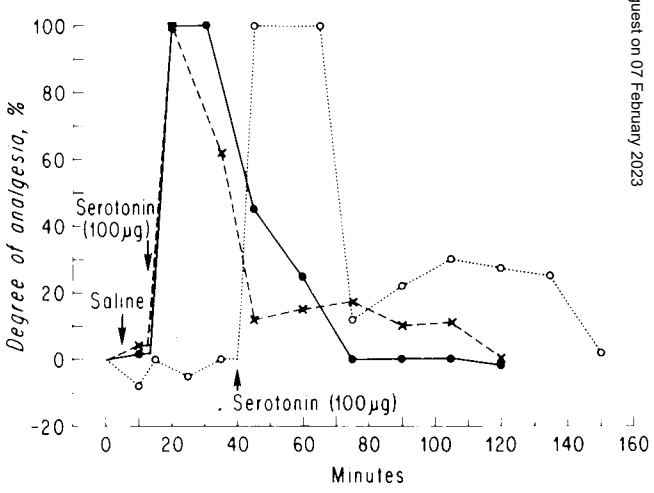

FIG. 3. Changes in degree of analgesia (serotonin, 100 μg).

leased. Furthermore, one can never be certain whether the response to injected serotonin is caused by an action on pathways normally involved in the function being observed or even whether the injected serotonin acts only at serotonin receptors.

The hypothesis that serotonin neurons may mediate sensitivity or reactivity to nociceptive stimuli has been substantiated further by the findings that the long-term consumption by rats of a tryptophan-poor corn diet decreases electroshock response thresholds. This hyperalgesia appears to be related directly to diet-induced decreases in the brain concentrations of serotonin.[12,13] Intracerebroventricular injection of serotonin potentiates the antinociceptive activity of narcotic agonists in experimental animals.[14]

Exact knowledge of the nature and localization of serotonin receptors is still lacking. Although serotonin depletion could antagonize the stimulation-produced analgesia,[8,9] naloxone administration has also been shown to antagonize part of the same analgesic effect.[15] Recently, Yaksh and Rudy[16] reported that morphine administered directly into the spinal subarachnoid space of the rat (acting only at the spinal level) produces potent analgesia that can be antagonized by naloxone. Because serotonin probably does not possess an affinity to opiate receptors,[17] serotonin and morphine may affect separate groups of receptors. The existence of opiate receptors and an endogenous morphine-like factor was suggested.[18] There may be different pathways to inhibit the nociceptive transmission at the spinal cord and in the higher central nervous system.

The existence of the modulating mechanisms of nociception may suggest that the organism has a built-in system to maintain optimal sensitivity to painful stimuli. Certain imbalances of the neurotransmitters may result in abnormal sensitivity to pain.

It is tempting to speculate that intrathecal administration of serotonin, morphine, or opiate-like factors may provide effective relief in certain pain syndromes. The advantage of this approach would be specific pain control without attendant loss of neurologic function. Further studies are needed to assess the analgesic effect and potential side effects of these substances.

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