($p < 0.001$), whereas in 90-day-old rats the increase was only 16% ($p < 0.05$). Constriction of the microvessels was minimal in 30-day-old rats at 5%, whereas in 90-day-old animals it was 30% ($p < 0.05$).

To give a general estimate of the age changes in sensitivity of the wall of the pial microvessels to laser irradiation, it can be said that their reactivity diminishes with age.

Local changes in the pial microcirculation were thus demonstrated in response to laser irradiation. The vessels of the microcirculatory bed differ in their sensitivity to laser irradiation. The intensity of the reaction depends on the duration of exposure, the diameter of the microvessel, and the animal's age. With age both the degree of magnitude of the response of the microvessels and its latent period change.

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EFFECT OF 5,7-DIHYDROXYTRYPTAMINE ON NOCICEPTIVE SENSITIVITY AND ON THE ANALGESIC EFFECT OF MORPHINE

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The role of serotoninergic brain mechanisms in the regulation of nociceptive sensation and the analgesic action of opiates remains the subject of vigorous discussion. Ideas on the importance of serotoninergic brain structures in the integration of nociceptive responses of different modalities and on correlation between the morphological state of the serotoninergic systems and changes in opiate analgesia are contradictory [5, 6, 13]. The participation of this neurotransmitter system in the formation of the hemodynamic manifestations of pain and their resistance to the action of morphine-like analgesics is virtually unstudied.

We therefore decided to study changes in nociceptive sensation, in pressor nociceptive responses of the arterial blood pressure (BP) and the analgesic effect of morphine in nociceptive tests with different levels of integration in the brain, after selective pharmacological destruction of serotoninergic systems.

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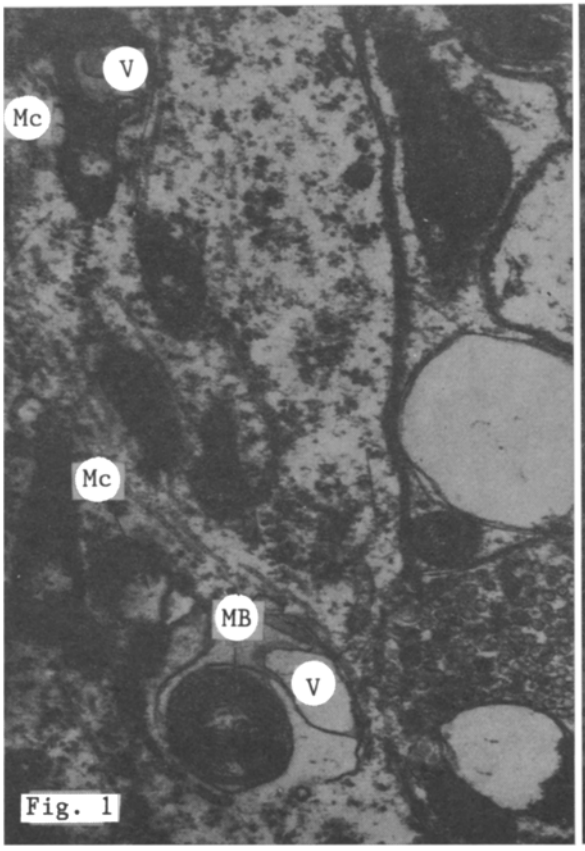

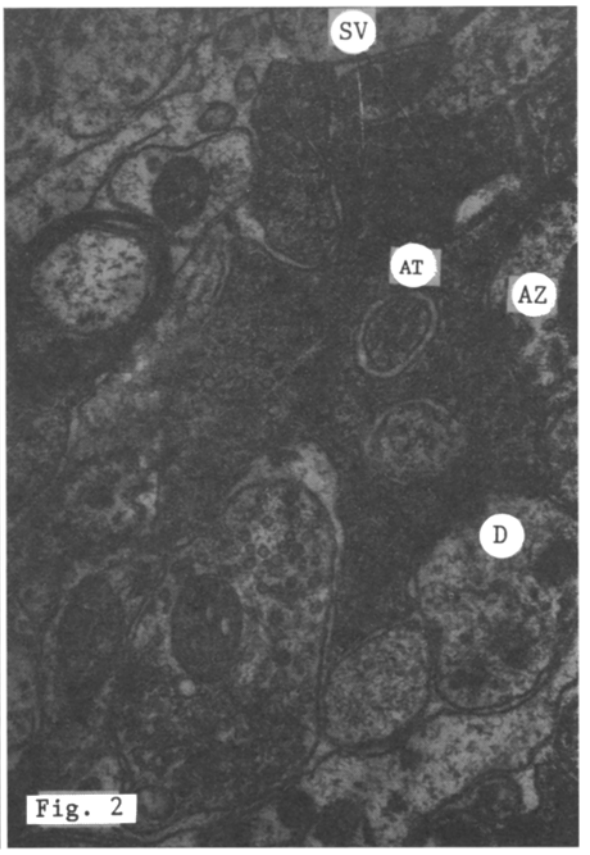

Fig. 1. Vacuolation of cytoplasm, fragmentation of mitochondria, and formation of myelin-like bodies in neuron of dorsal nucleus raphe 4 days after intracisternal injection of 5,7-DHT. Mc) Mitochondria; MB) myelinlike body; V) vacuole. 26,500 ×.

Fig. 2. Response of dark type of a large axon terminal forming divergent synapses in gray matter of posterior horn of spinal cord (T10) 2 days after injection of neurotoxin. AT) Axon terminal; SV) synaptic vesicles; AZ) active zone of synapse; D) dendrite. 37,500 ×.

EXPERIMENTAL METHOD

Experiments were carried out on 72 male Wistar rats into which desipramine (from "Serva", Switzerland) a selective blocker of high-affinity catecholamine uptake, was injected intraperitoneally 1 h before the operation, in a dose of 25 mg/kg. Later, under pentobarbital anesthesia (40 mg/kg), 200 µg of 5,7-dihydroxytryptamine (5,7-DHT; from "Serva"), a selective neurotoxin of serotoninergic mediation, was injected intracisternally in a volume of 10 µl. The brain and spinal cord of some of the rats (36) were fixed 1, 2, 5, 7, and 16 days after the injection of 5,7-DHT with 2.5% glutaraldehyde solution in phosphate buffer, by perfusion through the aorta, followed by postfixation of fragments of the brain in the same glutaraldehyde solution and in 2% osmium tetroxide solution in cacodylate buffer. Subsequent processing of the material for electron microscopy followed the standard procedure. The dorsal and central nuclei raphe and the dorsal and central regions of the posterior horns at levels of spinal segments C4-5 and T9-10 were investigated. Ultrathin sections were examined in Hitachi HU-11B and JEM-100B electron microscopes with a voltage of 75 kV.

Nociceptive sensation and the analgesic action of morphine (2 mg/kg, intraperitoneally) were studied in another group of animals (also 36) at the same times after injection of 5,7-DHT in the tail-flick and vocalization tests. At the same time BP and its pressor nociceptive changes were recorded in the carotid artery by a VI-6 5MA electromanometer. The control consisted of 12 rats undergoing mock operations and receiving intracisternal injections of 10 µl of physiological saline.

TABLE 1. Changes in Sensitivity to Pain, BP and Its Nociceptive Changes, and in the Analgesic Action of Morphine (2 mg/kg) under the Influence of 5,7-DHT

Experimental conditions	Latent period of tail-flick, sec	Vocalization threshold, μA	BP, mm Hg	Pressor changes of BP, mm Hg
Intact rats:				
control	13,1	0,44	115	27
morphine	17,3*	0,76*	107*	30*
7-8 days after injection of 5,7-DHT				
control	18,5*	0,40	86*	13*
morphine	16,4	0,40**	99**	10**
14-16 days after injection				
control	25,2*	0,40	111	14*
morphine	32,6**	0,38**	110	10**

Legend. *$p < 0.05$ difference significant between control and intact animals; **$p < 0.05$ differences significant between effect of morphine and intact animals.

EXPERIMENTAL RESULTS

Structural changes of compensatory, adaptive, and destructive nature were found in neurons of the dorsal and central nuclei raphe as early as 24 h after injection of 5,7-DHT. Vacuolation of the mitochondria and myelin-like bodies surrounded by a halo of electron-translucent cytoplasm, were found in the cytoplasm of these cells (Fig. 1). It is noteworthy that neurons with peripheral chromatolysis and vacuolation of the cytoplasm were found after 5 and 7, and even 16 days. Translucency of the dendroplasm and lysis and vacuolation of the microtubules were observed in the dendrites. Vacuoles and myelin-like bodies were found and the structure of the mitochondria was changed. Some of these organelles became electron-dense whereas others had a translucent matrix with fragmentation of the cristae. Changes of dark type with fusion of synaptic vesicles in the center of the terminal, and with a glial reaction developed in the region of the altered axons and terminals. At the same time, dendrites and axons with an ultrastructure similar to that of cells of the control animals, were found in the neuropil of the nuclei raphe.

Regions of accumulation of altered and degenerating axon terminals were discovered in the posterior horns of the spinal cord of the rats 2 days after injection of 5,7-DHT, but later their number was reduced. Characteristically the morphological features of the altered terminals differed. Terminals of average and large size contained many pale round synaptic vesicles of various sizes, or less frequently, single granular vesicles. Synapses formed by terminals of this kind (Fig. 2) were located as a rule on small branches of dendrites, a distinguishing feature of which was that synapses were found not only with dendrites, but also with boutons terminaux, probably belonging to a different mediator. Synapses of this kind can be regarded as the structural basis of presynaptic modulation [3]. Small terminals contained tiny pale round, ellipsoid, and flat synaptic vesicles in large numbers. These terminals were in contact mainly with the trunks of initial dendrites and with bodies of nerve cells. The structure and location of these synapses corresponded to those of inhibitory GABA-ergic synapses [12], suggesting close interaction between the GABA-ergic and serotoninergic systems in the posterior horns of the spinal cord.

Data on changes in sensitivity to pain, in nociceptive responses of BP, and the analgesic action of morphine after injection of 5,7-DHT are shown in Table 1. It was found that 5,7-DHT caused virtually no change in the vocalization threshold, but considerably increased the latent period of tail-flick and significantly reduced the pressor changes in BP, in association with moderate hypotension, which disappeared after 14-16 days.

The differences found in changes in sensitivity to pain after destruction of neurons of the nuclei raphe and their projections at first sight contradict the established views on

the hypoalgesic effect arising following deletion or destruction of the serotoninergic system [6], although it has recently been shown that inhibition of serotoninergic mediation leads to different changes in nociceptive responses, as regards the levels of their integration in the brain [5]. The absence of changes in vocalization thresholds is evidence, in our view, of the unimportant role of serotoninergic neurons of the nuclei raphe and their ascending terminals in the formation of this nociceptive reaction, in good agreement with existing data on the predominantly descending projection of the axons of these cells [4]. Hypoalgesia in the tail-flick test, developing after injection of 5,7-DHT simultaneously with destructive changes in neurons of the nuclei raphe and in their descending terminals, is evidently unconnected with changes in the motor sphere, for like other investigators [11], we did not find any hyperreactivity in the rats, and the very small decrease in muscle tone had already completely disappeared when sensitivity to pain was reduced (Table 1). The analgesia which we found in the tail-flick test, which is a predominantly segmental reaction [9], confirms the validity of the new hypothesis [13] on the existence of two functionally different descending serotonin systems. One of them facilitates the segmental nociceptive afferent input and segmental nociceptive responses, whereas the other inhibits activity of nociceptive neurons which have ascending projections. It is probably the first system that is represented by medium-sized and large descending terminals. Destruction of these terminals by neurotoxin leads to abolition of the descending facilitatory influences and to hypoalgesia, which can be detected in the test with a segmental reaction.

The analgesic action of morphine after injection of 5,7-DHT also showed different changes in the tail-flick and vocalization tests. Weakening of the effect of morphine in the vocalization test is in agreement with existing data [6] and is evidence of the important role of serotoninergic mediation in the realization of morphine analgesia at the suprasegmental level of the brain [7, 10]. Meanwhile the analgesic effect of morphine was unchanged in the tail-flick test, which also is in agreement with data obtained in recent years [5], and is compatible with the hypothesis put forward by Zemlan and co-workers [13]. Potentiation of the analgesic action of morphine 16 days after injection of 5,7-DHT may be due to its direct effect on the spinal cord, where it increases serotonin synthesis on account of its action of opiate receptors [7] against the background of hypersensitivity of the serotonin receptors, due to abolition of the descending serotonin-containing terminals [8].

Our experimental results are evidence of the essential importance of serotoninergic mediation in the regulation of nociceptive changes in BP also, which have as their morphological basis the intensive serotoninergic input to preganglionic neurons [8]. It is important to note that against the background of destructive changes in the nuclei raphe and descending serotonin terminals and reduction of the nociceptive changes in BP, morphine had a "paradoxical" action, manifested as still further weakening of these BP responses. The results suggest that serotoninergic mechanisms may play a key role in the realization of the sympathicoactivating action of morphine and morphine-like analgesics recently discovered by the writers [1, 2].

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