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Changes in Nociception After 6-Hydroxydopamine Lesions of Descending Catecholaminergic Pathways in Mice

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FASMER, O. B., O.-G. BERGE, L. TVEITEN AND K. HOLE. *Changes in nociception after 6-hydroxydopamine lesions of descending catecholaminergic pathways in mice.* PHARMACOL BIOCHEM BEHAV **24**(5) 1441–1444, 1986.—Intrathecal (ITH) administration of 5 μg 6-hydroxydopamine (6-OHDA) in mice selectively lesioned descending catecholaminergic pathways. Uptake of ^{3}H-noradrenaline (^{3}H-NA) into synaptosomes from the lumbar spinal cord was reduced by 95%, without any change in the uptake of ^{14}C-5-hydroxytryptamine (^{14}C-5-HT). Synaptosomal uptake of ^{3}H-NA and ^{14}C-5-HT in the brain was not altered. The nociceptive sensitivity was evaluated using the tail-flick, hot plate and formalin tests 3 and 14 days after injection of 6-OHDA. At day 3 hyperalgesia was found in the hot-plate test, unchanged response latency in the tail-flick test and hypoalgesia in the formalin test. At day 14 there were no statistically significant differences from controls in any of the tests. The present findings support the contention that catecholaminergic pathways participate in the tonic regulation of nociception in the spinal cord. However, while supraspinally integrated responses to acute thermal pain, as measured with the hot-plate test, are inhibited by these pathways, responses to prolonged chemical pain are enhanced.

Catecholamines 6-Hydroxydopamine Nociception Spinal cord

IT is well documented that descending catecholaminergic pathways are able to modulate the transmission of nociceptive information in the spinal cord. Intrathecally applied noradrenaline (NA) is a potent inhibitor of behavioural responses to noxious stimulation [17,23], and there is also evidence for tonic regulation of nociceptive sensitivity by spinal catecholaminergic pathways [14, 21, 22]. However, it is unclear whether these systems exert a general inhibition on nociceptive sensitivity at the spinal level or only affect the processing of certain types of nociceptive information [13,18].

In the present investigation changes in nociception after ITH injection in mice of the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) were studied. Intraventricular administration of 6-OHDA has been reported to cause degeneration of catecholaminergic nerve terminals in mice [20] as well as in rats [6]. To study different aspects of nociception, the tail-flick, hot-plate and formalin tests were employed. By using these tests it is possible to compare different types of noxious stimuli (thermal vs. chemical), different levels of integration of the behavioural responses (spinal vs. supraspinal) and different durations of the noxious stimuli. Behavioural testing was performed 3 and 14 days after injection of 6-OHDA, in order to assess both the actue effects of lesioning and the possible functional recovery. Evaluation of the lesions was made by measuring the synaptosomal uptake of ^{3}H-NA and ^{14}C-5-HT in the spinal cord and brain.

METHOD

Animals

Male albino mice (NMRI, 30–40 g) were used. The animals were housed in colony cages with free access to food and water. Testing took place during the light period of a 12/12 hr light-dark cycle. Each animal was tested only once, either in the hot-plate, tail-flick or formalin test. Separate groups of animals were used for biochemical analyses.

Intrathecal Injections

The intrathecal injection procedure was adapted from the method described by Hylden and Wilcox [15]. The animals were anaesthetized with a combination of pentobarbital (23 mg/kg) and chloral hydrate (100 mg/kg), and an incision was made in the skin to expose the vertebral column. The lumbar puncture was performed using a 30 gauge needle connected to a microsyringe with polyethylene tubing. The needle was inserted between L5 and L6, and 6-OHDA (5 μg, calculated as the base, Sigma) was injected in a volume of 10 μl. The control groups received an injection of vehicle (0.9% NaCl containing 0.2 mg/ml ascorbic acid).

Biochemical Analyses

The animals were killed by cervical dislocation, 14 days after the intrathecal injection of 6-OHDA or vehicle. The forebrain (rostral to the superior colliculi) and spinal cord

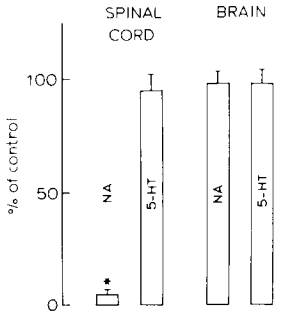

FIG. 1. Uptake of ^{3}H-noradrenaline (NA) and ^{14}C-5-hydroxytryptamine (5-HT) in synaptosomes from spinal cord and forebrain in 6-hydroxydopamine-treated mice (n=8). Mean±SEM. Data are given as percentage of the values in the vehicle injected control group (n=8). *$p<0.01$, Student's t-test.

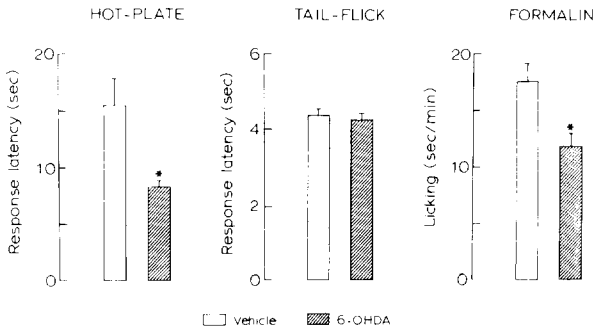

FIG. 2. Nociceptive sensitivity measured with the hot-plate, tail-flick and formalin tests 3 days after intrathecal administration of 6-hydroxydopamine (6-OHDA) (n=10–14) or vehicle (n=10–16). In the formalin test paw-licking was recorded from 0–10 min after injection of formalin. *$p<0.05$, Student's t-test.

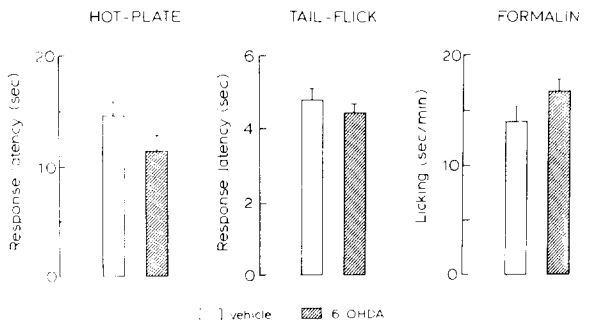

FIG. 3. Nociceptive sensitivity measured with the hot-plate, tail-flick and formalin tests 14 days after intrathecal administration of 6-hydroxydopamine (6-OHDA) (n=10–18) or vehicle (n=9–16). In the formalin test paw-licking was recorded from 0–10 min after injection of formalin.

(caudal to the 10th thoracic vertebra) were immediately removed and dissected on ice. Crude synaptosomes were prepared from the forebrain (approximately 300 mg) and spinal cord (approximately 50 mg). The tissue was homogenized in 10 vol. of 0.25 M sucrose. The homogenate was centrifuged (1000 × g, 0°C, 10 min), and 75 μl of synaptosome-containing supernatant added to a modified Krebs-Ringer bicarbonate buffer [12] to make a final volume of 0.7 ml. After preincubation at 37°C for 3 min, ^{14}C-5-HT creatinine sulphate (58 mCi/mmol, The Radiochemical Centre, Amersham) and ^{3}H-NA hydrochloride (14.8 Ci/mmol) was added to give a final concentration of 10 nM of the NA isotope and 100 nM of the 5-HT isotope. Incubation was continued for another 10 minutes and terminated by filtration through pre-washed GF/B glass microfiber filter (Whatman) by means of a cell harvester (Titertek, Flow Laboratories). The filters were washed for 15 sec with ice-cold saline and transferred to counting vials. Scintillation fluid (Insta-Gel II, Packard; 4 ml) was added. The samples were analysed in a Packard Tri-Carb 460 CD liquid scintillation spectrometer. Each determination was carried out in triplicate. Uptake determined in the presence of cocaine (1 mM) was subtracted from the total uptake. ^{14}C-5-HT and ^{3}H-NA activities were separated as described by others [25].

Testing of Nociception

The hot-plate test was performed using an IITC Inc. Mod. 35-D Analgesiameter set to a temperature of 55±0.2°C. Response criterion was licking of a hindpaw.

For tail-flick testing an IITC. Inc. Mod. 33 Analgesiameter was used [7]. The light beam was focused on the tip of the tail, and the intensity adjusted to give reaction times of 4–5 sec in the control groups.

The animals which were to be used in the tail-flick or hot-plate test were placed individually in standard macrolone cages (30×12×13 cm) one hour before testing.

The formalin test was modified from the method described by Dubusson and Dennis [10]. Before injection of formalin the mice were placed in individual cages which served as observation chambers. After one hour of adaptation to the cage, 20 μl of 5% formalin was injected into the dorsal surface of the right hindpaw. Assessment of pain intensity was performed by recording the amount of time the animals spent licking the injected paw during the first 10 min after the injection of formalin.

Testing of nociception was performed 3 and 14 days after ITH administration of 6-OHDA, in separate groups of animals. Results are given as mean±SEM.

Statistics

Student's t-test (two-tailed) was used to determine significant differences between groups ($p<0.05$).

RESULTS

Biochemical Analyses

Fourteen days after the intrathecal injection of 6-OHDA the synaptosomal uptake of ^{3}H-NA in the spinal cord was reduced to 5% of controls ($p<0.01$, Fig. 1). The uptake of ^{14}C-5-HT in the spinal cord and the uptake of ^{3}H-NA and ^{14}C-5-HT in the forebrain were not changed.

Changes in Nociception

Intrathecal administration of 6-OHDA 3 days before testing produced a significant hyperalgesia in the hot-plate test (Fig. 2). Response latencies were reduced to 53% of controls ($p<0.01$). Tail-flick latencies were not changed (96% of controls, Fig. 2). In contrast, hypoalgesia was found in the for-

malin test (Fig. 2). The amount of licking was reduced to 71% of controls ($p<0.05$).

Fourteen days following injection of 6-OHDA the hot-plate response latencies and the amount of licking in the formalin test were not significantly different from controls (Fig. 3). The tail-flick latencies were again unchanged.

DISCUSSION

The results of the present study have shown that it is possible, by ITH administration of 6-OHDA, to obtain a selective and extensive lesion of descending catecholaminergic pathways in mice. The synaptosomal uptake of ^{3}H-NA in the spinal cord was reduced by 95% 14 days after injection of 6-OHDA. The spinal 5-HT system was not affected, and there was no sign of damage to ascending catecholaminergic or serotonergic pathways, as evaluated by the synaptosomal uptake of ^{3}H-NA and ^{14}C-5-HT.

Most of the catecholaminergic fibres in the spinal cord are noradrenergic, but in addition there is a small dopaminergic pathway which accounts for approximately 10% of the catecholaminergic innervation [26]. It is not possible to ascertain to what extent spinal dopaminergic fibres were lesioned in the present study.

The contention that there is a tonic regulation of nociception by descending catecholaminergic neurones [21] was supported. Tonic inhibition was found when the hot-plate test was used, but in the formalin test tonic enhancement of nociception was demonstrated. Response latencies in the tail-flick test were not altered. Lesioning of descending catecholaminergic fibres by ITH 6-OHDA in rats has been reported to lower response thresholds both in the tail-flick and hot-plate tests [14,22]. Furthermore, ITH injection of the α-antagonist phentolamine produced hyperalgesia in both tests [21]. In other studies in rats, however, reduced hot-plate latencies, but unchanged tail-flik latencies were found after ITH 6-OHDA [13,18]. Similarly, intraventricular 6-OHDA in mice produced hyperalgesia in the hot-plate test without affecting tail-flick latencies [27]. On the other hand, selective lesioning of noradrenergic pathways in rats by systemic administration of the neurotoxin DSP4 failed to alter nociceptive responses in the tail-flick and hot-plate tests in rats [3]. The results reported in the literature are thus divergent, but the present findings support the contention that disruption of descending catecholamineric pathways affect response latencies in the hot-plate test, but not in the tail-flick test.

Systemic injection of the α-antagonist yohimbine or the β-antagonist propanolol in rats reduced pain responding in the formalin test [9]. These findings are in agreement with the results of the present study, which has shown that reduced catecholaminergic neurotransmission in the spinal cord elicits hypoalgesia in the formalin test.

The difference between the hot-plate, tail-flick and formalin tests are in the nature of the pain stimulus employed, in the duration of the stimulation and in the behavioural responses elicited. The tail-flick test measures a spinally integrated reflex response to radiant heat [16]. In both the hot-plate and the formalin tests hindpaw lick is used as response criterion, and thus requires supraspinal integration. In the tail-flick and hot-plate tests thermal stimuli of comparatively brief duration are employed, while in the formalin test chemical irritation produces a prolonged noxious stimulation. The neural mechanisms underlying the analgesic effects of morphine may be different in the tail-flick and formalin tests [1]. Furthermore, systemic administration of drugs that change monoaminergic function has been shown to alter morphine analgesia differently in the tail-flick, hot-plate and formalin tests [8,9]. The findings of the present study have shown that disruption of catecholaminergic neurotransmission in the spinal cord produced different results in these tests. This emphasizes the importance of using more than one test-method to evaluate changes in nociception.

Taken together with the results of other investigations, the present findings indicate that tonic activity of descending catecholaminergic pathways is only exerted on supraspinally integrated behavioural responses to noxious stimuli, and does not affect spinal nociceptive reflexes. The opposite results in the hot-plate and formalin tests indicate that responses to acute thermal pain are inhibited, while responses to prolonged chemical pain are enhanced. On the basis of the present study it is not possible to decide if the difference between the two tests is caused by different nociceptive stimuli being used, or is related to the different duration of the stimuli.

It has previously been reported that changes in nociceptive sensitivity after lesioning of descending catecholaminergic pathways disappear after 14 days [22]. These observations were confirmed in the present study. Similar results have also been found after lesioning of descending serotonergic fibres with ITH 5,6-dihydroxytryptamine in mice [11] and rats [4]. It is known that regeneration of central noradrenergic fibres takes place after neurotoxic lesions [5]. However, it is unlikely that this is the explanation for the observed compensation process in the present model. The biochemical determinations were performed on day 14 and showed an extensive damage to spinal catecholaminergic terminals. The compensatory processes may be related to the development of supersensitivity of postsynaptic receptors, to increased turnover in the remaining catecholaminergic terminals or to other transmitter systems taking over the function of the lesioned fibres [24].

In conclusion, the present study has shown that lesioning of descending catecholaminergic pathways alters responses to noxious stimuli in the hot-plate and formalin tests, but not in the tail-flick test. The contention that catecholaminergic pathways participate in the tonic regulation of nociception in the spinal cord is thus supported. These pathways tonically inhibit nociceptive sensitivity recorded with the hot-plate test, but tonically enhance the behavioural responses to pain induced by formalin. Mechanisms involved in the spinal modulation of nociception thus may be different for different types of pain.

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