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Analgesia Induced by Neonatal Capsaicin Treatment in Rats

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SAUMET, J.-L. AND R. DUCLAUX. *Analgesia induced by neonatal capsaicin treatment in rats.* PHARMAC. BIOCHEM. BEHAV. **16**(2) 241–243, 1982.—Twenty-seven rats were treated by 475 mg kg^{-1} of subcutaneous capsaicin from 2 to 7 days of their life. After treatment, only 11 rats had survived. Their cutaneous heat, pressure, pricking and visceral hyperosmolarity nociceptive thresholds were measured between 50 and 170 days after treatment. The results showed a considerable, or even total, analgesia to cutaneous as well as to visceral stimuli. The conclusion is that same pathways carried all the nociceptive messages independently of nature or localization of noxious stimuli.

Capsaicin Nociceptive stimuli Analgesia

JANCSÓ *et al.* [6, 9, 10] have shown that the nociceptive chemical sensitivity of the skin was irreversibly abolished in rats neonatally treated by capsaicin, the pungent agent of the red pepper. These rats showed no reduction in sensitivity to physical stimulation: i.e. mechanical or thermal stimuli [8]. For Jancsó *et al.*, these results suggest that the pathways for chemonociception are specifically destroyed by capsaicin while the pathways for thermal or mechanical pain remain intact. However, this interpretation of the effect of capsaicin does not seem to be confirmed by recent results. In neonatally treated rats Holzer *et al.* [5], Nagy *et al.* [12], Hill *et al.* [4] showed that the sensitivity to nociceptive cutaneous heat stimuli was decreased. Furthermore, the neonatally capsaicin treated rats, tested by Hill *et al.* [4] were insensitive to mechanical nociceptive stimuli. These results suggest that capsaicin may act indistinctly on all the nociceptive pathways. Therefore, it may be supposed that its action is independent of the nature or of the localization of the nociceptive stimuli. In order to verify this hypothesis we have measured the effect of neonatal administered capsaicin on nociceptive stimuli of various nature including heat, pressure, pricking and hyperosmotic solution and of various localizations including cutaneous and visceral nociceptive stimulations.

METHOD

Capsaicin Injections

Twenty-seven Wistar rats, two days old, received five subcutaneous injections of capsaicin (Merck) up to a total dose of 475 mg kg^{-1}. Capsaicin was solubilized in ethanol (10%), twin 80 (10%), and isotonic saline solution (80%). The rats were injected once a day from the second day until the 7th day of their life. They received 25 mg kg^{-1} the first and 50, 100, 100, 200 mg kg^{-1} on the following days.

Nociceptive Responses Measurement

The response to noxious stimuli was measured when the treated rats were from 50 to 170 days old. Date of birth of control rats was unknown but they had approximately the same weight as treated rats. Nociceptive stimuli were thermal, mechanical and chemical. Cutaneous and visceral nociceptive sensations were tested. Thermal and mechanical stimuli were applied on the skin of the tail, while the chemical stimulus was applied intraperitoneally.

Heat Stimuli

The noxious radiant heat threshold was measured by the tail flick test described by D'Amour and Smith [3]. A radiant beam is focussed on the tails of rats. In response to burning, the rat moves its tail and frees the orifice of photo-electric cells which stops an electric timer. The duration of the tail reaction measured the burning nociceptive threshold. The same day, tail flick latency was measured six times on each of the 12 control rats. Results are expressed as the mean of these six measurements. Tail flick latency was measured only once in the 11 treated rats because they showed cutaneous lesions after a single test. Tests were applied at 50th and 80th days of their life.

Mechanical Stimuli

Two mechanical stimuli were applied on the tail: nociceptive pressure with a blunt pusher and nociceptive pricking with a needle. The mechanical thresholds were measured, using a "Ugo Basile" analgesimeter, by the weight applied to the blunt pusher or the needle which provoked either vocalizations or removal of the tail. Each one of 10 treated rats and 12 control rats were tested six times. The results are expressed as the mean of these measurements. Treated rats were 73 days old.

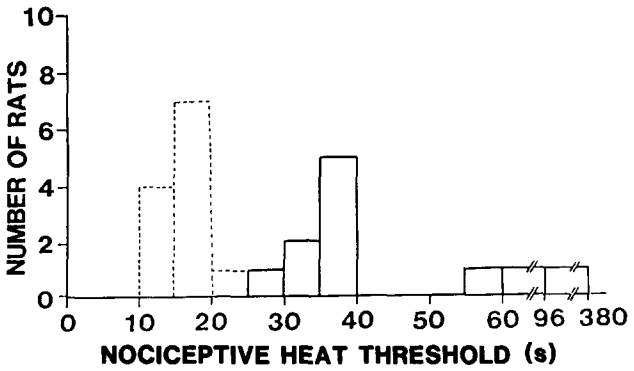

FIG. 1. Nociceptive heat thresholds determined by tail flick latency in 11 neonatal capsaicin treated rats —— and 12 control ---.

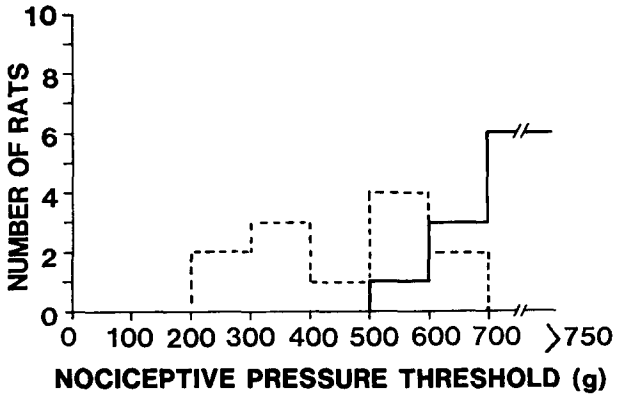

FIG. 2. Nociceptive pressure thresholds in 10 neonatal capsaicin treated rats —— and 12 control rats ---.

Chemical Stimuli

Visceral stimuli consisted of an intraperitoneal injection of saline hypertonic solution at 7.3 osm in six treated rats, 180 days old. In normal rats the acute visceral pain produced by the injection provoked a vocalization, a limping walk, drawn in sides and an inhibition of natural behaviors such as exploration and grooming. These 5 criteria were taken as a means of measuring visceral nociception. Moreover, the injection of hypertonic saline solution induced a drinking behavior in response to the hyperosmolarity of the extracellular fluid [2]. In our experiment, the bottle was on the top of the cage and the rats were obliged to stand up on their hind legs in order to drink. We observed that this behavior was inhibited by visceral pain. Latency between injection and drinking was therefore used as a measurement of visceral pain. Drinking latency was recorded as visceral nociception measurement.

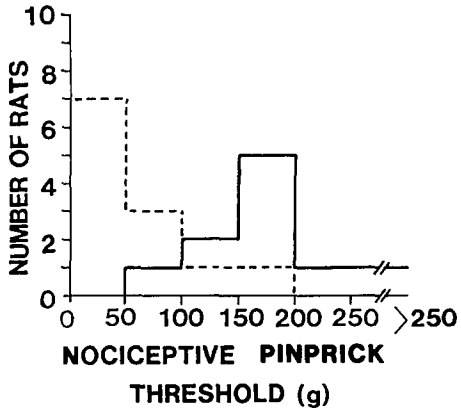

FIG. 3. Nociceptive pinprick thresholds in 10 neonatal capsaicin treated rats —— and 12 control rats ---.

RESULTS

Lethality of Neonatal Capsaicin Treatment

On 27 capsaicin treated rats, only 11 rats had survived. Sixteen rats died during treatment. No obvious difference between the 11 surviving treated rats and the control rats was observed as far as appearance, behavior or motility are concerned.

Cutaneous Nociceptive Responses

Nociceptive Heat Threshold

In 12 control rats, tail flick latency ranged between 7 and 20 sec with a mean of 11.33 sec. In treated rats, 50 days old, the tail flick latency was significantly increased as compared to control (Fig. 1). After measurement (Fig. 1) cutaneous inflammation was visible on the tail. Only 3 out of 11 treated rats removed their tail under test. Latency was respectively 25.9 sec, 30 sec, 33.6 sec (Fig. 1). The other 8 treated rats did not react at all to burning (Fig. 1). In order to avoid permanent lesions, radiant heat was manually stopped after a time ranging between 40 to 95.5 sec for 7 rats (Fig. 1). Radiant heat was left on for 375.6 sec in one rat, but no movement or vocalization was observed. The 10 others were retested at 80 days of their life; these showed a persisting analgesia to burning since radiant heat did not provoke removal of tail or vocalization.

Nociceptive Mechanical Threshold

Response to pressure. Figure 2 shows that 6 treated rats, 73 days old, were insensible to nociceptive pressure i.e. they did not remove their tail or vocalize when the maximum weight of the algesimeter (750 g) was applied on their tail. Four treated rats removed their tail, but three had a threshold higher than the majority of the control group (Fig. 2).

Response to pricking. Figure 3 showed that one treated rat was insensitive and others had a low sensitivity to nociceptive pricking ranging between 100 and 250 g with a mean of 159.5 g (Fig. 3). Controls had thresholds ranging between 5 and 200 g with a mean of 56.9 g (Fig. 3).

Response to Visceral Chemonociceptive Stimulation

Table 1 shows that the neonatally treated rats had an considerable decrease of the nociceptive visceral sensitivity to hypertonic saline solution. The results of the 7 tests appear in Table 1. The difference between control and treated

TABLE 1

RESPONSE ARE SHOWN FOR 30 MIN AFTER ONE INTRAPERITONEAL INJECTION OF SALINE HYPERTONIC SERUM IN SIX NEONATALLY CAPSAICIN TREATED RATS (T) AND SIX CONTROL RATS (C)

	Control n=6	Treated n=6
Vocalization	yes	no
Duration of drawn in sides (mn)	24.3	4.5
Aspect of drawn in sides	considerable	small
Duration of limping walking (mn)	22.6	0
Latency of exploration (mn)	23	1.3
Latency of grooming (mn)	19.9	3.6
Latency of drinking (mn)	26.3	11.5

Each time is in minutes (mn).
Aspect of drawn-in sides was described as small or considerable.

rats was obvious in all tests. After injection treated rats manifested either no, or very weak, visceral pain symptoms such as vocalization, drawn in sides, lumping walk. Drinking induced by the hypertonicity of the saline solution was delayed only in control rats (Table 1). Furthermore, exploration and grooming were inhibited in controls only (Table 1).

DISCUSSION

Neonatal capsaicin treatment decreased or even abolished the sensitivity to nociceptive stimuli independently of their nature or localization. This confirmed previous studies. Cutaneous heat nociceptive thresholds were increased by neonatal capsaicin injection (Nagy *et al.* [12], Holzer *et al.* [5], Hill *et al.* [4]). Moreover, Hill *et al.* [4] showed that pretreated rats were insensitive to pricking, and Jancsó *et al.* [10] have already shown a decreased nociceptive chemosensitivity of the skin and the cornea. Our study extends these previous results to visceral nociception, which was also abolished by capsaicin pretreatment. Furthermore, in the present experiment analgesia was total or subtotal. Jancsó has previously shown such intense analgesia, but only for chemonociception. The observation of a total analgesia may be explained by cumulative dose of capsaicin used in the present experiment (475 mg/kg) as compared with 50 mg/kg used in other studies [4, 5, 8, 12].

Since no change of motility was observed in neonatal capsaicin treated rats in Holzer [5] and our study, it may be concluded that this drug is not toxic for all neurons; however its neurotoxic action is not specific of pain since it also modifies thermoregulation [1, 7, 13, 15]. Several studies have shown that capsaicin treatment acts on the primary sensory afferents. Cutaneous applications of capsaicin produced a local cutaneous analgesia in humans [10]. Morphological results of Jancsó *et al.* [8], and Scadding [14] showed a destruction of 64 and 70 percent of amyelinic fibers in saphenous nerve of rats treated by neonatal capsaicin injection. Moreover, the results of Jessel *et al.* [11] suggest that capsaicin produces selective depletion of substance P in primary sensory endings in the substancia gelatinosa of spinal cord. Substance P could be a neuromediator for pain messages. The analgesic action of capsaicin administered in neonates may be interpreted as showing that peripheral nociceptive neurons subserving pain of different natures and localizations possess common metabolic or structural system specificly destroyed by capsaicin. However, the peripheral action of capsaicin does not exclude a central action in nociception. Indeed, it has been shown that the mitochondria of hypothalamic neurons are modified by capsaicin treatment [15].

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