platelet MAO activity reflects monoaminergic activity in the tubero-infundibular system.

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9. The volunteers included 38 whites and 1 black (23 males and 16 females) ranging in age from 18 to 32 years.
10. The lowest decile of MAO activity for males ranged from 2.40 to 7.98 nmole of benzaldehyde produced per 10^8 platelets per hour. For females the range was from 7.14 to 10.47 nmole of benzaldehyde per 10^8 platelets per hour.
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12. Platelet MAO activity for the highest decile of males and females ranged from 12.57 to 21.54 and 14.92 to 22.22 nmole of benzaldehyde produced per 10^8 platelets per hour, respectively.
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14. The patient group included 15 whites and 4 blacks (14 males and 5 females) ranging in age from 20 to 45 years.
15. Median prolactin concentrations were used rather than mean values to prevent occasional spuriously high values from exerting an unduly large effect.
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Intrathecal Capsaicin Depletes Substance P in the Rat Spinal Cord and Produces Prolonged Thermal Analgesia

Abstract. *A single intrathecal injection of capsaicin depletes substance P from primary sensory neurons and causes a prolonged increase in the thermal and chemical pain thresholds of the rat but no apparent change in responses to noxious mechanical stimuli.*

Substance P may play a role in the transmission of noxious stimuli at primary afferent synapses in the dorsal horn (*1*). The peptide is located within synaptic vesicles in primary afferent terminals (*2*) and has been observed within neurons of small diameter within the dorsal root ganglion (*3*). The iontophoretic application of substance P excites those neurons that respond to noxious peripheral stimuli (*4*). Recently, we reported that substance P is released in vivo from the cat spinal cord by high-intensity, but not low-intensity stimulation of the sciatic nerve (*5*). Administration of the homovanillic acid derivative, capsaicin, into the spinal perfusate of rats and cats produces a calcium-dependent release of substance P (*5*); whereas repeated subcutaneous administration of capsaicin reduces the concentration of substance P in the dorsal horn (*6*). In addition, systematically administered capsaicin has been reported to alter the response of peripheral nerves to chemical stimuli applied to the skin and to block the animal's thermoregulatory response (*7*).

These observations led us to investigate whether animals treated with capsaicin might display analgesia. To restrict the actions of capsaicin to the spinal cord, we administered the drug directly into the subarachnoid space. In rats under ether anesthesia we implanted a polyethylene catheter by inserting it through the cisterna magna to the rostral edge of the lumbar enlargement in the spinal subarachnoid space (*8*). The rats were allowed to recover for 7 days, after which time they each received an intrathecal injection of 3 or 30 μg of capsaicin in a volume of 15μl (*9*) and then an injection of 10 μl of saline.

Intrathecal injection of capsaicin produced a striking biphasic response. During the first 1 to 3 minutes, the animal showed a strong contracture of the caudal portions of the body; after this period the animals regained coordinated motor control and immediately began biting and scratching at the caudal portion of the body in the dermatomes corresponding to those levels of the spinal cord affected by the intrathecal injection. This severe agitation lasted 5 to 10 minutes, after which the animal sat quietly showing occasional grooming behavior.

Twenty-four hours after the injection of capsaicin, we tested the nociceptive threshold by using the spinally mediated tail-flick response to heat and by measuring the withdrawal latency on a 55°C hot plate. In animals tested before injection of capsaicin, the mean latency on the

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tail-flick test was 1.9 ± 0.4 seconds (mean ± standard error; $N = 20$) and the mean latency on the hot plate was 14 ± 2 seconds ($N = 20$). Injections of capsaicin produced a block of the tail-flick and hot plate response. Most of the animals (70 to 80 percent) treated with high doses of capsaicin exhibited no escape response in either test (Fig. 1). In contrast to the control rats, capsaicin-treated rats showed no reduction in thermal analgesia when tested repeatedly at intervals up to 5 months after a single intrathecal injection of 30 μg of capsaicin (Fig. 1).

To assess the behavioral specificity of the sensory deprivation induced by capsaicin, we compared the responses of rats to a number of different sensory stimuli. Application of a 70°C thermal probe to the caudal region of the back near the midline failed to elicit characteristic skin twitch responses in rats treated with capsaicin. The injection of 0.05 ml of 10 percent formalin solution in the left hind paw normally produces a sequence of behaviors in which the animal favors the hind paw and frequently licks it. In capsaicin-treated rats this behavior was significantly reduced (*10*). The writhing response induced by intraperitoneally administered phenylquinone, was also reduced in capsaicin-treated animals (*11*).

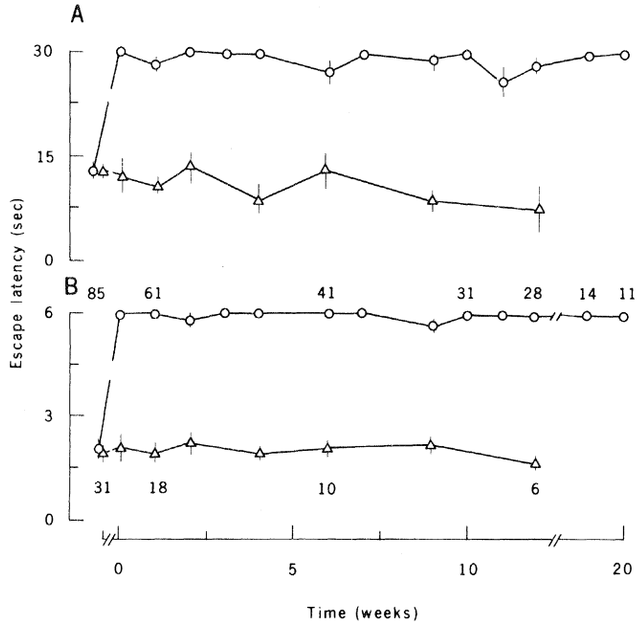

Fig. 1. Mean latencies (± standard error) of responses to (A) hot plate and (B) tail-flick for two groups of rats before and over a period of up to 5 months after receiving either 30 μg of capsaicin (○) or vehicle (△). In (B) the numbers at each point indicate the number of animals whose data are included in the hot plate and tail-flick measure at that time. The reduced numbers over time reflect the loss of animals through normal attrition.

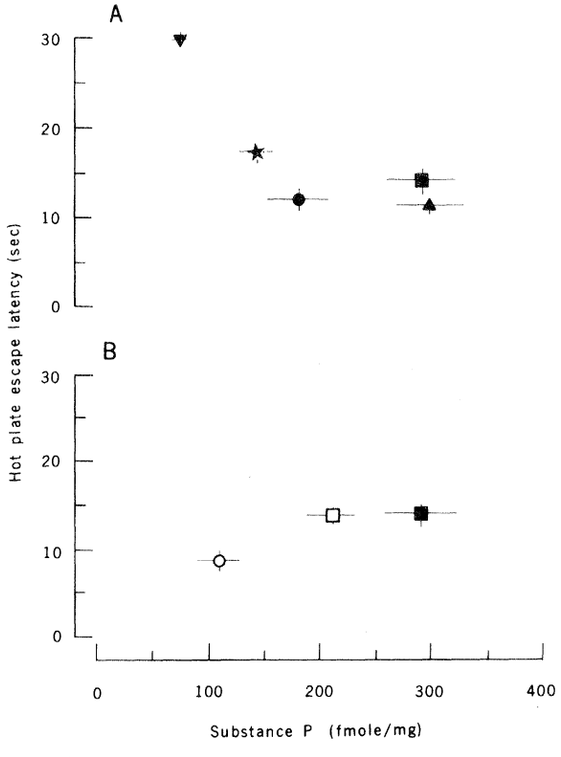

Fig. 2. (A) Analgesia measured on the hot plate as a function of the concentration of substance P in the spinal cord of rats at the time of death after intrathecal injection of ▼, 30 μg of capsaicin ($N = 7$); ★, 3 μg of capsaicin ($N = 3$); or ●, DMSO vehicle ($N = 5$) or intravenous injection of ▲, 30 μg of capsaicin ($N = 6$). Controls are shown by ■ ($N = 8$). (B) Similar data for rats given an intrathecal injection of ○, 20 μg of 5,6-dihydroxytryptamine ($N = 10$); or □, ascorbic acid vehicle ($N = 5$). Controls are shown by ■ ($N = 8$).

Assessment of the motor capacity of capsaicin-treated animals displaying analgesia revealed no significant alteration in motor function. Rotorod testing failed to distinguish among rats without implanted catheters and vehicle- or capsaicin-treated animals. The animals remained able to climb a vertical wire mesh, exhibited normal righting, stepping, and placing reflexes, and responded normally in tests of the orientation response to light touch and the startle jerk associated with a puff of air directed at the flank. Although quantitative measurement of the response to noxious pinch is difficult to assess, there was no discernable difference in the agitation-withdrawal response or the degree of squeaking between capsaicin- and vehicle-treated animals upon application of forceps to the dorsum of the hind paws or the base of the tail. Thus, the profound thermal and chemical analgesia was not associated with any detectable alteration in response to mechanical stimuli or in loss of motor coordination.

In a separate series of experiments, rats were given a single intrathecal injection of either 3 or 30 μg of capsaicin and the nociceptive threshold was assessed on day 7, at which time most of the animals were unresponsive in both the tail flick and hot plate tests. The animals were then killed, the spinal cord (lumbar region) was rapidly removed, and the concentration of substance P was measured by radioimmunoassay (*12*). Implantation of the catheter alone produced some depletion of substance P, but this was unassociated with any change in response latency. In animals treated with intrathecal capsaicin (30 μg), there was a 55 percent reduction ($P < .01$) in the concentration of substance P in the lumbar spinal cord (Fig. 2) compared to vehicle-treated controls. Animals with little depletion of substance P did not show increased nociceptive thresholds, and injection of 3 μg of capsaicin produced a 17 percent reduction in substance P with only a minimal increase in nociceptive threshold (Fig. 2).

Substance P within the spinal cord is located predominantly in the terminals of primary afferent neurons (*3*); however, there is a significant contribution from some raphe-spinal neurons in which substance P appears to coexist within sero-

tonin (*13*). To investigate the possibility that capsaicin might elicit thermal analgesia by depleting substance P within descending raphe-spinal neurons, we destroyed the spinal terminals of raphe-spinal neurons by the intrathecal injection of 20 μg of 5,6-dihydroxytryptamine (5,6-DHT). Seven days later we measured the animals' responses to the hot plate and tail-flick tests, then killed them and measured substance P in their spinal cords (*14*). Intrathecal injections of 5,6-DHT produced a 50 percent depletion in the substance P content of the lumbar spinal cord; however, this depletion was not associated with analgesia. These animals, in fact, exhibited a significant decrease in nociceptive threshold ($P < .05$) (Fig. 2B).

Intrathecal injection of capsaicin produced no significant change in the substance P of the brainstem or the forebrain. In addition, intraventricular injection of capsaicin (40 μg in 20 μl) did not produce analgesia. It is known that capsaicin exerts profound peripheral actions, producing intense pain when applied peripherally and causing the activation of C fibers in peripheral nerve (*7*). Capsaicin (30 μg) given via the tail vein evoked no agitation and no changes in the nociceptive threshold up to 7 days after injection (Fig. 2A). Spinal substance P concentrations in animals given capsaicin intravenously did not differ from those in control animals without implanted catheters (Fig. 2A). These experiments suggest that thermal and chemical analgesia evoked by intrathecal capsaicin is not mediated by the diffusion of the drug to either supraspinal sites or the peripheral nervous system.

How does capsaicin produce prolonged thermal and chemical analgesia? We do not believe the effect to be related to the general alteration in spinal function. Animals treated with intrathecal capsaicin maintained good signs of motor function as well as normal responses to non-noxious peripheral stimuli and to noxious mechanical stimuli applied to the extremities; such responses were qualitatively similar to those of control animals. Treated animals also showed no signs of self-mutilation which characteristically accompanies rhizotomies in the rat (*15*), suggesting that capsaicin does not produce a chemical axotomy. The levels of serotonin and norepinephrine in the spinal cord are unchanged by intrathecal capsaicin (*16*), whereas glutamic acid decarboxylase activity and opiate receptor binding are unaffected by systemically administered capsaicin at doses sufficient to deplete substance P (*6*). Capsaicin-produced analgesia was not abolished by administration of the opiate antagonist naloxone (2 mg/kg), and although intrathecal injection of morphine elicits analgesia, the effect is of short duration (4 to 6 hours). Furthermore, intrathecal opiates produce analgesia not only to thermal and chemically induced pain, but also to noxious mechanical stimuli (*8, 17*). Thus, capsaicin-elicited analgesia seems independent of endogenous opiate systems.

We propose that the analgesia resulting from intrathecal capsaicin may be the consequence of a series of events in which capsaicin rapidly liberates most releasable stores of substance P from primary afferent terminals and then induces a prolonged and possibly permanent depletion of substance P from primary sensory neurons associated with the transmission of thermal noxious stimuli. From these studies it is not clear whether the effect of capsaicin is restricted to small-diameter chemo- and thermoreceptive primary afferents. Capsaicin may also deplete other components that play a role in the transmission of nociceptive information. Although the present experiments cannot be construed as supporting a clinical use for capsaicin, the ability to deplete, in a specific manner, a nociceptive transmitter at the level of the first-order afferent synapse may represent the future direction of research for improved analgetic therapy.

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9. Capsaicin was dissolved in 50 percent dimethylsulfoxide and saline. All control injections consisted of this vehicle except where noted.
10. Under double-blind conditions, the degree of agitation in rats was assessed on a scale of 1 to 4 every 5 minutes for a period of 2 hours. The mean rating score for vehicle-treated animals in the first and second hour was 3.4 ± 0.4 and 2.5 ± 0.6, respectively ($N = 5$); in capsaicin-treated animals, the same measures were 1.7 ± 0.2 and 0.8 ± 0.3 ($N = 8$). When we used the sign test, the capsaicin group showed a significantly lower ($P < .01$) agitation rating than untreated controls during each hour.
11. Phenylquinone, 0.25 ml of 0.02 percent alcohol solution, was injected intraperitoneally and the animals housed in individual plastic cages. The number of times each animal rubbed its belly on the floor (writhes), was accumulated. The mean number of phenylquinone-induced writhes in vehicle-treated animals was 32 ± 6 ($N = 6$) compared to 6 ± 2 ($N = 6$) in the capsaicin-treated animals ($P < .01$; sign test).
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14. This treatment causes a 70 to 90 percent depletion of cord serotonin at 7 days with no changes in brainstem concentrations of 5-hydroxytryptamine (5-HT) or any changes in either brainstem or spinal concentrations of norepinephrine. The depletion of spinal 5-HT is associated with a reduction in the nociceptive threshold which is thought to reflect the loss of a descending modulatory influence (T. L. Yaksh and G. M. Tyce, and H. Proudfit and T. L. Yaksh, unpublished observations).
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18. T.M.J. is a Harkness Fellow. Supported by grant NS 14629 from the National Institute of Neurological and Communicative Disorders and Stroke and grant DA-02110 from the National Institute for Drug Abuse to T.L.Y.

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Stereospecific Sorption of L Amino Acids by Colloidal Clay

The statement by Bondy and Harrington (*1*) that "the stereospecificity of [clay-organic reactions] has not been investigated" is incorrect. In 1971 I reported data demonstrating preferential adsorption and polymerization of L isomers of amino acids relative to D isomers and DL mixtures by kaolinite crystals (*2, 3*); a preliminary note on my findings was published by Degens *et al.* (*4*). The results were tentatively ascribed to the inherently enantiomorphic crystal structure of the clay.

Several other investigators have attempted comparable experiments, the results being positive in some cases (*1, 5,*

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