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Inhibition of acute inflammation in the periphery by central action of salicylates

(central nervous system modulation of inflammation/indomethacin/dexamethasone)

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ABSTRACT **Understanding of the antiinflammatory actions of nonsteroidal drugs is incomplete, but these actions are believed to occur in the periphery, without any contribution from the central nervous system. Recent research on the antipyretic antiinflammatory neuropeptide α-melanocyte-stimulating hormone indicates that it can act centrally to inhibit peripheral inflammation; this raises the possibility that other agents, such as nonsteroidal antiinflammatory drugs, may have similar activity. In the present research both lysine acetylsalicylate and sodium salicylate inhibited edema, induced in the mouse ear by topical application of picryl chloride, when injected into the lateral cerebral ventricle. This inhibitory activity on a measure of acute inflammation was not due to escape of the drugs into the periphery, because systemic injection of doses that were effective centrally did not affect inflammation. In contrast, central administration of a dose of indomethacin that was antiinflammatory when given intraperitoneally did not inhibit peripheral inflammation. Thus indomethacin apparently lacks the central antiinflammatory action of the salicylates. This observation, plus our inability to demonstrate either an antiinflammatory effect of intracerebroventricular dexamethasone, a prostaglandin inhibitor, or a pro-inflammatory influence of prostaglandin E_2, suggests that prostaglandins are not important to central modulation of inflammation. The results indicate that, in addition to having central influences on fever and pain, salicylates can act within the brain to inhibit acute inflammation in the periphery.**

Hypotheses about the mechanisms of action of antiinflammatory drugs have focused on peripheral actions such as inhibition of hydrolytic enzymes, metabolism of arachidonic acid, and migration of polymorphonuclear leukocytes, monocytes, and lymphocytes into injured tissue (1). More recently, local cytokines such as tumor necrosis factor and interleukin 1 have been linked to inflammation (2). Although the central nervous system (CNS) was identified as a possible target for the action of nonsteroidal antiinflammatory drugs (NSAIDs) in pioneering studies (3), modern reviews of antiinflammatory agents do not mention any influence of the CNS (e.g., refs. 4 and 5). However, recent observations indicate that peripheral acute inflammation characterized by edema can be inhibited by a central action of the neuropeptide α-melanocyte-stimulating hormone (α-MSH; melanotropin); local application of picryl chloride to the mouse ear evoked edema that was inhibited in a dose-dependent fashion by intracerebroventricular (i.c.v.) administration of this peptide (6). The effect on edema could not be traced to an α-MSH-induced increase in circulating corticosterone, a glucocorticoid that has marked antiinflammatory activity. α-MSH, like common antipyretic drugs, also reduces fever when given centrally or peripherally (7).

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The antipyretic action of these common drugs is believed to differ from their antiinflammatory action in that it must occur within the brain (8). However, because (*i*) α-MSH and antipyretic/antiinflammatory drugs have similar effects; (*ii*) the drugs, whether given centrally or peripherally, act within the brain to inhibit fever; and (*iii*) as stated above, central injection of α-MSH inhibits acute inflammation in the periphery, it may be that NSAIDs likewise act centrally to influence inflammation. To test this idea, NSAIDs were administered i.c.v. to mice with acute cutaneous inflammation induced by local application of picryl chloride.

MATERIALS AND METHODS

Female BALB/c mice (Simonsen Laboratories, Gilroy, CA), 7 weeks old, were housed at 23–25°C (range) in groups not exceeding five per cage (28 cm long × 18 cm wide × 13 cm high). They were acclimatized under standard conditions for at least 1 week with food and water freely available. Several shipments of mice were required for these experiments, and the base-line inflammatory response to picryl chloride is known to differ slightly among animals from different shipments. For this reason, tests of specific agents were performed as separate experiments in which experimental and control animals in each study were drawn from the same shipment.

Each animal was anesthetized with 10% pentobarbital sodium solution (50 mg/kg; Nembutal; Abbott). Base-line thickness of both ears was measured with a spring-loaded micrometer (Swiss Precision Instruments, Los Angeles). To induce ear edema, a classic sign of acute inflammation, both sides of each ear were coated with 10 μl (40 μl total per mouse) of 0.5% picryl chloride in acetone (9). Immediately thereafter, an NSAID, dexamethasone, or prostaglandin E_2 (PGE_2), dissolved in nonpyrogenic saline (20 μl), or saline alone (20 μl), was injected directly into a lateral cerebral ventricle of each anesthetized mouse. The general technique has been described previously (10). The animals were anesthetized again 3 and 6 hr later, and ear swelling was determined by subtracting the base-line thickness from the measurements obtained for each ear at 3 and 6 hr. The differences for the two ears were averaged for the final analysis. The NSAIDs lysine acetylsalicylate (Maggioni-Winthrop, Milan), sodium salicylate (Fisher Scientific), and indomethacin were tested. The salicylates were selected both because of their potent antiinflammatory activity and because they are soluble in water and can therefore be readily injected into the brain. A water-soluble form of indomethacin, sodium indo-

Abbreviations: CNS, central nervous system; NSAIDs, nonsteroidal antiinflammatory drugs; α-MSH, α-melanocyte-stimulating hormone (α-melanotropin); i.c.v., intracerebroventricular(ly); PGE_2, prostaglandin E_2.

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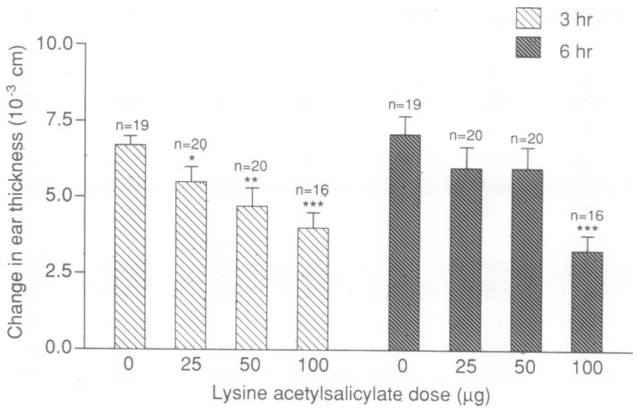

FIG. 1. Inhibition of peripheral edema by centrally administered lysine acetylsalicylate. Scores in this and following figures are mean ± SEM changes in ear thickness. Number of animals (n) is given above each bar. The probability refers to comparisons with control values at the same time after administration of the agent: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$.

methacin trihydrate (courtesy of C. M. Stemmler, Merck Sharp & Dohme), was tested for the same reasons and because of its marked inhibitory effect on prostaglandin synthesis. Dexamethasone and PGE_2 were obtained from Sigma.

Analysis of variance techniques (Dynastat, Philadelphia) were used to test for significant overall differences in separate analyses for each agent. The Student–Newman–Keuls test was used to compare specific differences in the mean effects of control and drug treatments.

RESULTS

Lysine acetylsalicylate administered i.c.v. inhibited acute peripheral inflammation (Fig. 1, $F = 10.35$, $P < 0.0001$). Edema was inhibited in a dose-related fashion at 3 hr, with a mean maximum of 41% inhibition after the 100-μg dose; only this dose remained effective at 6 hr (53% inhibition). The antiinflammatory influence of central lysine acetylsalicylate was not secondary to escape of the drug into the periphery, because neither the largest effective central dose (100 μg) nor a dose 10-fold greater had any effect on inflammation when given i.p. (Fig. 2). On the other hand, a systemic dose of 10 mg did inhibit inflammation, perhaps consistent with sufficient penetration of the drug into the brain, as suggested from previous research on salicylate distribution in the mouse (11), and/or with adequate peripheral action of the drug.

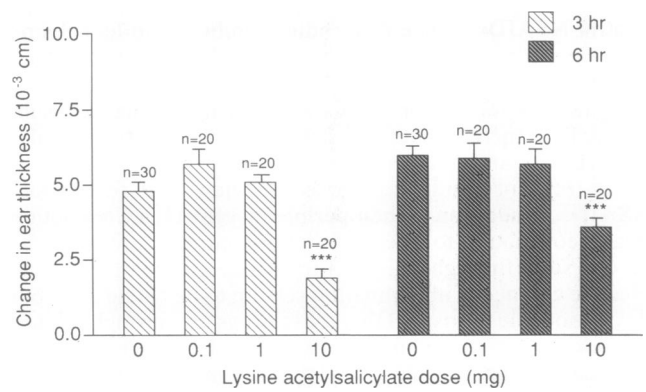

FIG. 2. Injection i.p. of the most effective central dose of lysine acetylsalicylate (0.1 mg) had no effect on edema, nor did a dose 10-fold greater (1.0 mg). A dose 100-fold greater inhibited inflammation.

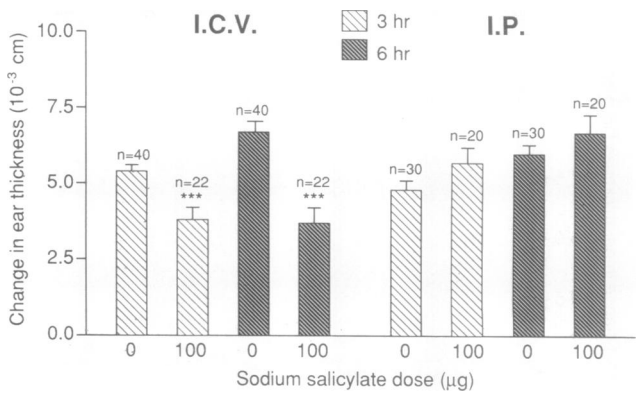

FIG. 3. Sodium salicylate (100 μg) inhibited inflammation when given i.c.v. but not when given i.p.

To learn whether the central antiinflammatory effect is limited to the specific molecular conformation of lysine acetylsalicylate, 100 μg of sodium salicylate was administered both i.c.v. and i.p. (Fig. 3). Given centrally, this agent likewise inhibited acute inflammation of the ear ($F = 38.9$, $P < 0.0001$). The inhibition of edema was approximately 30% at 3 hr and 44% at 6 hr. As with lysine acetylsalicylate, i.p. administration of the same dose had no influence ($F = 3.19$, $P = 0.89$).

To test further the generality of the central action of NSAIDs on acute inflammation, indomethacin was administered centrally. A dose of 100 μg had no effect on edema (Fig. 4). One milligram was not only ineffective, it killed two of five mice in initial tests. When 100 μg of indomethacin was given i.p., it did inhibit the edema ($F = 18.1$, $P < 0.0001$). Inhibition was 51% at 3 hr and 42% at 6 hr.

Dexamethasone, a potent synthetic glucocorticoid believed to inhibit inflammation locally, in part by inhibition of arachidonic acid release, was administered in a dose judged to be over 6-fold more effective than a prednisolone dose that had previously been shown, when given i.p., to inhibit inflammation in the mouse ear (9). Dexamethasone had no inhibitory action on peripheral edema when injected i.c.v. (Fig. 5). Rather, there was a small but significant increase in ear swelling at 3 hr.

To test the idea raised by the indomethacin and dexamethasone results that central prostaglandins are not important to modulation of peripheral inflammation, PGE_2 or saline was injected i.c.v. after picryl chloride treatment in 40 mice. Doses of 0.25, 0.5, and 1.0 μg ($n = 10$ for each dose) had no effect on peripheral inflammation ($F = 0.4$, $P = 0.76$); the

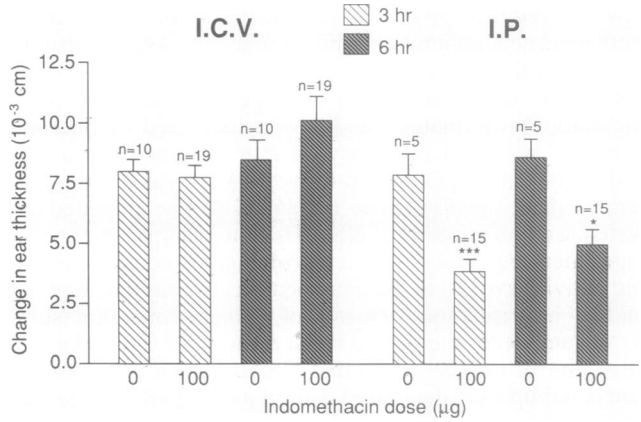

FIG. 4. Indomethacin (100 μg) had no effect on edema when given i.c.v., but it inhibited the acute inflammation after i.p. injection.

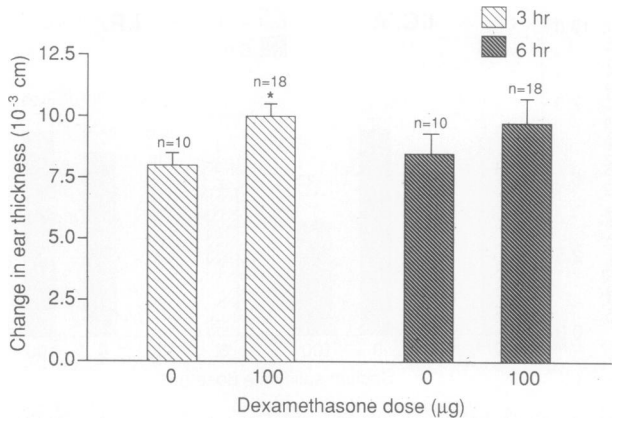

FIG. 5. Dexamethasone (100 μg) given i.c.v. did not inhibit peripheral inflammation.

range of mean percent change was −4% to +3% for 3-hr readings, −7% to +4% for 6-hr readings.

DISCUSSION

The antiinflammatory actions of NSAIDs are not fully understood, but explanations for their actions have focused on events in the periphery (1). Perhaps the strongest theory is that such agents act by inhibiting peripheral prostaglandin synthesis (see ref. 12). However, there is no close correlation between the capacity of NSAIDs to inhibit synthesis of prostaglandins and their capacity to reduce inflammation. Our results suggest an alternative explanation that may account in some part for the effects of salicylates: modulation of peripheral inflammation via actions within the brain. It is clear that such drugs act centrally to reduce fever (8, 13), and salicylate has been shown to reach CNS sites in substantial amounts (11). This evidence, coupled with the present data, suggests an antiinflammatory influence of such drugs that is mediated centrally. These findings do not eliminate the possibility that NSAIDs act both within the brain and peripherally to reduce inflammation; rather, they link with previous results to support this conclusion.

The central effect of NSAIDs on edema of acute inflammation appears to parallel their central influences on pain. Although Lim and his associates (14, 15) suggested that nonnarcotic analgesic drugs had no central mechanism of action, there is more recent evidence that such agents do reduce pain via central actions. Ferreira *et al.* (16) found that hyperalgesia induced by injection of carrageenan into the rat paw was reduced by central administration of aspirin, indomethacin, acetaminophen, and phenacetin. Aspirin also altered central electrical activity induced in humans by painful stimulation of the teeth (17). The pain threshold to tooth stimulation in primates was likewise increased by microinjection of sodium acetylsalicylate into preoptic-anterior hypothalamic sites (18). Several investigators have noted that central pain signals in the thalamus can be inhibited by systemic administration of NSAIDs. For example, in a recent experiment by Braga (19), ketoprofen given centrally inhibited activity evoked in thalamic neurons by manipulating the ankle of arthritic rats. By means of C-fiber stimulation (sural nerve) and recording of neuronal activity in the thalamus, Jurna and Brune (20) found that NSAIDs such as indomethacin and ibuprofen decreased central pain signals. These and other studies provide substantial evidence that NSAIDs can act centrally to reduce pain. It is, therefore, not unreasonable to accept that drugs known to have antiinflammatory activity, as well as antipyretic and analgesic activity, after systemic administration exert this action, at least in part, within the CNS.

How do central NSAIDs reduce acute cutaneous inflammation? There is no definite answer to this question, but descending neuronal pathways may be involved. Inflammation has a neurogenic component in that peripheral terminals of primary nociceptive afferent neurons not only signal pain but are the source of inflammatory mediators, such as substance P (21), believed to be released via activity of nonmyelinated fibers of the sympathetic nervous system. There is recent evidence (22) that plasma extravasation induced in rats by activation of unmyelinated primary afferents, mast cells, or sympathetic postganglionic nerve terminals is significantly reduced by surgical excision of the lumbar sympathetic chain. Plasma extravasation is a major feature of swelling in the mouse ear edema model used in the present experiments. Furthermore, induction of an inflammatory response on one side of the body results in similar changes on the other side (23, 24); sciatic neurectomy attenuates the contralateral response. Selective lesions of small-diameter afferents or of postganglionic sympathetic efferents also retard and attenuate swelling in the uninjured paw (25), whereas venous ligation does not, ruling out a contribution of hormonal factors. Additional evidence of neurogenic involvement in inflammation are findings that rheumatoid arthritis is often bilateral and that rheumatic disease is often less in paretic limbs (26).

Given the existence of a neurogenic mechanism of inflammation, how might centrally acting NSAIDs influence it? As with the central actions of many drugs, the explanation is not clear. Inhibition of central prostaglandin synthesis is an unlikely step in the mechanism in light of the lack of influence of centrally administered indomethacin, a potent inhibitor of prostaglandin synthesis, on ear swelling in the present research. Further, dexamethasone, a potent peripheral antiinflammatory agent that inhibits arachidonic acid release, exhibited no central action. In addition, the lack of an action on peripheral inflammation of artificially increasing the central prostaglandin concentration argues against an important influence of central prostaglandin action in this model.

Modulation of lower circuits by supraspinal influences is a basic principle of nervous system function, and neurogenic aspects of inflammation may be modulated much as pain signals are modulated. Pathways descending from the periaqueductal gray (PAG) substance and nucleus raphe magnus (NRM) of the brainstem via the dorsolateral funiculus are known to modulate pain signals (27). There has been no attempt to test the effect of PAG or NRM stimulation on acute inflammation per se, but the relation between inflammation and pain in the periphery is strong. It may be that central NSAIDs induce descending inhibitory influences on the spinal cord, dorsal root ganglion, and sympathetic chain to reduce the neurogenic aspect of inflammation, perhaps via inhibition of release of agents such as histamine and substance P, or their precursors, that are known to alter vascular permeability and to cause pain.

The present results reinforce the view that for certain NSAIDs, in addition to their peripheral effects, a CNS action might contribute to their antiinflammatory activity. The precise steps through which central salicylates inhibit aspects of acute cutaneous inflammation remain to be elucidated, but it is reasonable to suspect that these drugs act through release of endogenous secondary mediators within the brain. One candidate for such a role as a secondary mediator is α-MSH, a neuropeptide recently shown to inhibit acute peripheral inflammation when administered centrally (6).

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