mammals, it seems possible that the diene analogs of **1** observed in the rat arose through biliary or intestinal (*13*) excretion of **1** or its metabolites (or both) from the intraperitoneal administration, followed by epoxide reduction in the intestine. Conversions of epoxide to olefin within the digestive tract may thus represent a significant metabolic pathway in mammals for potentially toxic epoxides, which include alkylating agents, carcinogens, and some pesticides. In the case of dieldrin, epoxide reduction is not a detoxication because the aldrin produced is itself toxic and its major metabolic transformation is epoxidation by liver oxidases back to dieldrin (*14*). However, reduction of the epoxide moiety in other compounds can be expected to diminish biological activity in cases where the olefin is not readily reepoxidized, or by allowing sufficient time for additional biodegradation to occur before reepoxidation. It also seems likely that reductions of epoxide to olefin in the digestive tract may function nutritionally in the reduction of oxidized foodstuffs such as fatty acid epoxides and cutin acid epoxides.

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Antagonism of Stimulation-Produced Analgesia by Naloxone, a Narcotic Antagonist

Abstract. *Analgesia produced by focal electrical stimulation of the brain is partially reversed by the narcotic antagonist naloxone. The absence of complete reversal does not appear to be caused by inadequate doses of naloxone since doses higher than 1 milligram per kilogram of body weight did not increase the antagonism. It is suggested that stimulation-produced analgesia may result, at least in part, from release of an endogenous, narcotic-like substance, such as that recently reported by other investigators.*

Focal electrical stimulation of the brain produces analgesia in the rat, the cat, and in man (*1–4*). This stimulation-produced analgesia exhibits several striking features in parallel with the analgesia produced by narcotic drugs. Both appear to exert their effects at sites surrounding the third ventricle, the cerebral aqueduct, and rostral portions of the fourth ventricle (*2, 5, 6*). Drugs that affect transmission in central monoamine pathways alter both morphine analgesia and stimulation-produced analgesia (*7, 8*). Morevoer, tolerance develops to the analgesic effect of brain stimulation and cross-tolerance between morphine and brain stimulation occurs (*9*). We report now that stimulation-produced analgesia can be partially blocked by the narcotic antagonist naloxone. This observation has important implications for the neural mechanisms of pain inhibition. A preliminary report of some of these findings has been made (*10*).

Forty-one male Sprague-Dawley rats were used. A single bipolar electrode constructed of twisted stainless steel wire (0.2 mm in diameter), Teflon-coated except at the cut cross section of the tips, was implanted in the periaqueductal gray matter, an area known to yield particularly potent and reliable analgesia (*1, 2, 11*). Analgesia was measured in a modified version of the D'Amour and Smith tail-flick test (*12*), in which one records the latency of the spinally mediated withdrawal reflex of the tail in response to the application of radiant heat. The apparatus and procedure have been described (*7*). The radiant heat source was adjusted to produce a baseline latency (BL) of 3.5 to 4.5 seconds. Following brain stimulation, if the animal did not respond within 7.0 seconds, the heat was automatically shut off in order to prevent tissue damage. The degree of analgesia (DA) due to brain stimulation was expressed as percentage and derived from the ratio of actual change in response time (T) from baseline to the maximum possible change according to a formula adapted from Benson *et al.* (*13*)

$$DA = 100\,(T - BL)/(7 - BL)$$

Following recovery from surgery, animals were screened to determine appropriate parameters of brain stimulation. Trains of 60-hertz sine-wave current 100 msec in duration were delivered at a rate of 3 per second for 20 seconds. For each animal, two current intensities were determined, one producing an intermediate degree of analgesia (30 to 60 percent), the other yielding total analgesia (100 percent). During screening, current intensity was raised in steps of 10 μa (peak-to-peak) until a 100 percent DA was observed. Current intensities above 200 μa were not employed. Once determined, current intensity was held constant throughout the experiment. Only animals exhibiting minimal motor and sensory side effects during stimulation were employed in these studies. In each test session, a BL was obtained by averaging three trials separated by 2-minute intervals. Analgesia was then assessed in three additional trials each immediately preceded by 20 seconds of brain stimulation. Since analgesia usually outlasted the period of brain stimulation, sufficient time was allotted between stimulation trials to permit pain responsiveness to return to prestimulation levels (normal BL).

For all experiments, animals were given three testing sessions, each separated by 2 days. These are referred to as predrug, drug, and postdrug sessions. In the predrug session, animals were injected with a matched volume of the naloxone vehicle (0.9 percent saline) and were tested 20 minutes later to obtain a BL score, then a DA score after brain stimulation. The drug session was identical except that animals received naloxone instead of vehicle. The postdrug session was identical to the predrug session.

In an initial experiment, nine animals were stimulated at an intensity yielding 100 percent DA. As shown in Fig. 1A, the predrug vehicle control did not affect analgesia. However, naloxone (1 mg per kilogram of body weight) reduced DA to a mean of 62 percent. Stimulation-produced analgesia was affected by naloxone in seven of the nine animals; and, in these, DA scores under the drug ranged between 7 and 89 percent. The DA was significantly higher in the postdrug session than in the drug session ($P < .05$, one-tailed t-test). In

this and in the other experiments, naloxone had no effect on baseline tail-flick latencies. It thus appears that, in our hands, naloxone causes a selective but partial disruption of the mechanisms underlying pain inhibition without affecting pain responsiveness per se (*14*).

Since in this first experiment the central stimulating current has been set to yield maximum (100 percent) analgesia, it is possible that some animals had been excessively stimulated and for this reason naloxone caused only a partial reversal. Therefore, in a second experiment, the stimulating current was adjusted to produce an intermediate degree of analgesia in the predrug session (58 percent mean DA for eight animals). As seen in Fig. 1B, naloxone (1 mg/kg) reduced the DA to 36 percent ($P < .005$, one-tailed t-test). The DA was significantly higher in the postdrug session than in the drug session ($P < .05$, one-tailed t-test). Once again, it appears that naloxone antagonizes stimulation-produced analgesia, but only partially. In fact, the relative block in analgesia caused by the drug (38 percent) was the same in both experiments.

Another possible explanation for the failure of naloxone to antagonize stimulation-produced analgesia completely is that an insufficient dose of the drug was administered. In an additional 24 animals, therefore, the effects of higher doses of naloxone (2 and 4 mg/kg) were assessed. These doses were tested both when the central stimulation current was set to give 100 percent as well as intermediate levels of analgesia. In each test, naloxone caused a statistically significant drop in DA, but in no case did a higher dose of naloxone produce a greater mean blockade of analgesia than did the 1 mg/kg dose. Thus, although complete reversal of analgesia by naloxone was not found with the doses employed in these experiments, a significant partial antagonism was consistently observed.

That naloxone can antagonize stimulation-produced analgesia supports strongly our earlier contention (*1, 2, 8, 15*) that similar mechanisms are involved in stimulation-produced and narcotic analgesia. An explanation for this phenomenon may lie in recent reports that there is an endogenous, morphine-like substance in the brain (*16–18*). This substance is found in high concentrations in areas overlapping those where stereospecific binding of opiates occurs (*17*). In the present study, analgesia was induced by stimulation of the periaqueductal gray matter, an area shown to contain a significant number of opiate binding sites (*6*). Thus, stimulation-produced analgesia may result, at least in part, from the release of a neurochemical modulator onto these binding

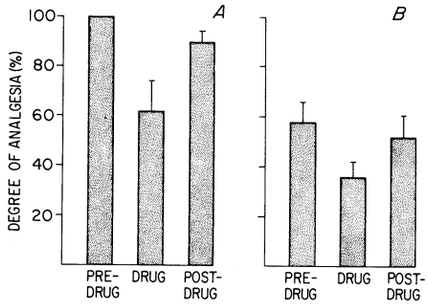

Fig. 1. The effects of naloxone (1 mg/kg) on stimulation-produced analgesia in (A) animals of experiment 1 showing an initial degree of analgesia of 100 percent and in (B) animals of experiment 2 showing a mean initial degree of analgesia of 58 percent. Vertical bars represent the standard error of the mean.

sites. Naloxone would antagonize the action of this substance at central receptor sites as it does in the mouse vas deferens (*16*). However, we have observed that the effect of naloxone on stimulation-produced analgesia is variable among animals and is usually incomplete. It is possible that the analgesic effect of brain stimulation does not result from the activation of a single underlying mechanism. Within or near the periaqueductal gray matter may lie other substrates of inhibition with which opiates and opiate antagonists do not interact. Moreover, stimulation of presynaptic elements in the periaqueductal gray matter might release the morphine-like substance onto postsynaptic receptor sites, and this effect would be completely antagonized by naloxone. But stimulation might also affect postsynaptic cells directly, and this effect would presumably be immune to naloxone action. In this regard, the reported tolerance to stimulation-produced analgesia was also incomplete (*9*). The degree of tolerance occurring in that study was quite similar to the degree of blockade produced by naloxone in the present work.

These studies suggest that there is a neural system in the brain which utilizes an endogenous, morphine-like substance to produce analgesia. It may be that activation of this system can be brought about either pharmacologically by direct receptor stimulation, or electrically by release of the endogenous substance. In either case, naloxone is seen to antagonize the analgesic effect. There is also evidence that other analgesic manipulations may utilize this system. For example, Mayer (*19*) has reported antagonism of acupuncture analgesia in man by naloxone.

Various narcotic-like effects may occur endogenously and participate in a variety of normal regulatory processes of the brain. Exogenously administered narcotic drugs may exaggerate these processes and lead to compensatory effects and problems associated with dependence. Yet, selective access to these systems by focal electrical stimulation of the brain may, in some circumstances at least, allow utilization of the beneficial aspects of their opiate-like action with minimal engagement of undesirable effects. Evidence that this approach is valuable in relieving intractable pain in man is already available (*4, 20*).

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