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5. The fieldworks were supported by the National Geographic Society, the Fundación M. Lillo, and the Universidad Nacional de Tucumán, Argentina. The specimens are part of the paleontological collection of the Fundación M. Lillo, Tucumán, Argentina.

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Morphine-Naloxone Interactions: A Role for Nonspecific Morphine Excitatory Effects in Withdrawal

Abstract. *The opiate antagonist naloxone precipitates withdrawal when given either 15 minutes after or 1 minute before a single injection of morphine in drug-naïve mice. We propose that withdrawal signs arise from a synergistic mixture of excitatory influences that are direct (agonistic action on nonspecific opiate receptors) and indirect (sensory and affective disorders, stress, hormonal and neurotransmitter dysfunction, and so forth). The predominant effects during precipitated withdrawal are assumed to be direct, whereas during abstinence in tolerant animals they are indirect.*

As much as 50 years ago, morphine was reported to be "at one and the same time a depressant and a stimulant" (*1*), with stimulation unexplainably evident after administration of single massive doses or after prolonged administration in which large doses are reached gradually. In recent years the excitatory nature of morphine has been documented in terms of acetylcholine turnover (*2*) and of increased impulse discharge of certain neurons, both after single doses (*3*) and during repeated administration (*4*). We have also been unable to explain convincingly the fact that during repeated administration, tolerance develops for the depressant action while hypersensitivity can develop to the excitatory action (*5*).

We report here data that we believe can permit these perplexing questions to be answered. The rationale for these experiments was developed in consequence of recent reports about morphine excitatory effects. Jacquet and colleagues demonstrated that morphine microinjection into the periaqueductal gray of drug-naïve rats could cause excitation in addition to the commonly seen catalepsy and analgesia; the excitation was not reversed by naloxone, a stereospecific antagonist (*6*). Such excitatory effects were mimicked by microinjection of *d*-morphine, which does not act stereospecifically at the opiate receptor (*7*). This excitatory response to morphine was similar to the behaviors seen in the withdrawal syndrome; it was suggested that precipitated abstinence could be due to a selective blockade of stereospecific receptors but not of nonspecific receptors (that is, receptors that are not blocked by naloxone).

This hypothesis is partially supported by the demonstration that naloxone, a drug generally presumed to cause a stereospecific blockade of opiate receptors, could precipitate withdrawal after only a single dose of morphine (*8*). However, withdrawal could not be demonstrated after morphine injection into an animal that had been first treated with naloxone (*9*). This was interpreted to mean that the nonspecific excitatory effects were not causing withdrawal.

Nonetheless, we believed that the previous failures to demonstrate a morphine role in withdrawal reactions were correctable by changes in experimental procedures. In our preliminary tests, several factors appeared to be critical:

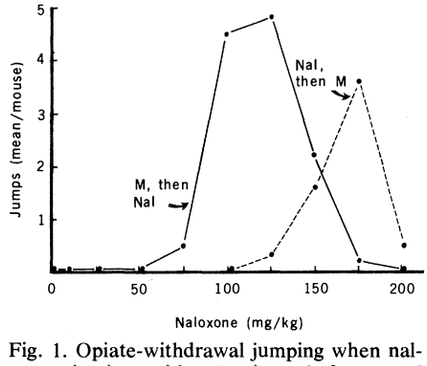

Fig. 1. Opiate-withdrawal jumping when naloxone is given either 1 minute before or 15 minutes after a single dose of morphine (50 mg/kg) in previously unexposed mice. The greater effectiveness of naloxone (*Nal*) when it is given after morphine (*M*) could indicate some rapid-onset tolerance within 15 minutes after morphine or could reflect the locomotor inhibitory effect of naloxone when it is given alone (before) morphine. Six mice were tested per data point; these same doses of naloxone given to saline-treated mice never produced jumping. Numbers of mice showing jumping at the optimal doses for both naloxone curves differed significantly from saline-injected controls ($P < .05$, chi-square test).

the ratio of morphine to naloxone dosage, the requirement for a large dose of naloxone, and the need to reduce the time interval between injections when naloxone was given first.

Subjects were female Texas-Swiss outbred mice, injected intraperitoneally with morphine and naloxone in various sequences and doses. Pilot studies indicated that a convenient morphine dose for this purpose was 50 mg per kilogram of body weight. Mice were housed communally with a constant number of mice per cage. All treatments were given between 1800 and 2200 hours. Behavioral signs of withdrawal that were evaluated are the commonly accepted signs of hyperactivity, hyperreactivity, repeated escape attempts, rearing and sniffing, and piloerection. However, the most objective and quantifiable sign, stereotypic jumping, was the prime index of precipitated withdrawal. Number of jumps for each mouse was scored during the first 15 minutes after the last drug injection; a jump was scored whenever a mouse cleared the wall of an opaque plastic dishpan 34 by 30 by 14 cm.

Initially, we evaluated precipitated withdrawal when morphine was administered first, followed in 15 minutes by naloxone in a full range of doses (left curve in Fig. 1). Naloxone reliably precipitated withdrawal symptoms at doses between 125 and 175 mg/kg. These doses are higher than those used by Jacob *et al.* (*9*, *10*). The inhibition of withdrawal by still higher doses of naloxone is presumed to reflect the importance of the agonist-antagonist ratio. For a given dose of morphine, only a certain range of naloxone was effective; this may also indicate other behavioral effects of naloxone.

Rapid development of tolerance to single doses of morphine occurs under certain conditions. To see if such a phenomenon could underlie our precipitated abstinence, we concluded a second series of tests in which naloxone was given first, followed 1 minute later by morphine. Using a similar dose range as before, we found that withdrawal signs were produced even under these conditions (right curve in Fig. 1). Lower doses of naloxone also precipitated withdrawal symptoms, but jumping occurred with less reliability. Jumping never occurred in saline-injected controls ($N = 6$ for each dose of naloxone).

High doses (> 25 mg/kg) of naloxone, given alone, caused apparent freeze behavior, with pronounced huddling in one corner of the cage and conspicuous lack of exploratory behavior. Such behaviors interfere with induction of jumping, but

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the problem was circumvented by injecting the following dose of morphine before the naloxone-induced behavioral inhibition became fully developed. At a lower naloxone dose (10 mg/kg) the interval between naloxone and morphine could be increased while still resulting in withdrawal signs other than jumping. We have focused on the jumping index of withdrawal because that is considered to be the most unequivocal indicator (*11*).

Thus we have shown that, irrespective of the sequence in which drugs were given, naloxone could precipitate withdrawal in nontolerant mice. Giving naloxone first did cause the dose-response curve to shift to the right (Fig. 1); the decreased effectiveness of naloxone could be due to the behavioral inhibition when naloxone was given first. Also, since testing was 0 to 15 minutes after morphine injection when naloxone was given first, compared to 15 to 30 minutes after morphine injection when morphine was given first, there could have been less morphine available to act on nonspecific excitatory receptors.

Both sets of data strongly support a role for nonspecific morphine effects in the appearance of withdrawal symptoms, at least with precipitated withdrawal; mechanisms may be different for the abstinence syndrome (*12*). Commonly, we presume that the appearance of these excitatory effects is prevented by the concurrent existence of morphine's depressant effects. A general model (Fig. 2) illustrates the various interrelationships and predicts the conditions under which naloxone-precipitated withdrawal can occur. The underlying assumptions of the model are that withdrawal signs appear because of a hyperexcitability state resulting from the synergistic effect of direct and indirect actions. Direct actions include an excitatory effect of morphine, which can only be expressed when stereospecific (depressive) receptors are blocked or inactivated because of tolerance; naloxone could also have some direct agonist action on nonspecific receptors. Indirect actions of naloxone derive mainly from its ability to block stereospecific, depressive receptors; this blockade prevents not only the depressive effect of morphine but also that of endorphins and enkephalins. Naloxone may also have indirect actions through putative amino acid transmitters (*13*).

The model predicts that precipitated withdrawal would occur whenever a certain concentration of morphine is available to cause direct excitation mediated by nonspecific receptors, in concert with the indirect effects of naloxone. The model also predicts that the time course or order of drug injections is not critical for demonstrating precipitated withdrawal, but that at lower doses the ratio of doses could be critical. The model explains the results from the laboratories of Jacquet (*6, 7*) and Jacob (*9*), and also explains why naloxone-precipitated withdrawal can be more severe than spontaneous withdrawal (*11*). Further, the model also helps to explain many heretofore puzzling phenomena, such as convulsions induced by large single doses of morphine (*1*) and the occasional failure of high doses of morphine to alleviate precipitated withdrawal symptoms in patients with a high degree of dependence (*14*).

Finally, the model is compatible with the probable mechanisms underlying spontaneous withdrawal in tolerant animals. In this instance, some contribution from direct excitatory effects could occur (as morphine levels are falling). However, the predominant excitatory influence would seem to be indirect, resulting from what might be called "disequilibrium shock" arising from a constellation of sensory, affective, stress, hormonal, neurotransmitter, and other influences (*15*) (including perhaps impaired endorphinergic transmission due to tolerance).

In summary, we believe that our data show that a significant contributor to precipitated withdrawal symptoms comes, not from the absence of opiate, but from its presence, in a limited range of concentrations, coupled with an appropriate degree of inactivation of stereospecific receptors. These direct excitatory effects also occur during sudden abstinence in tolerant animals, but in this situation indirect excitatory influences probably predominate.

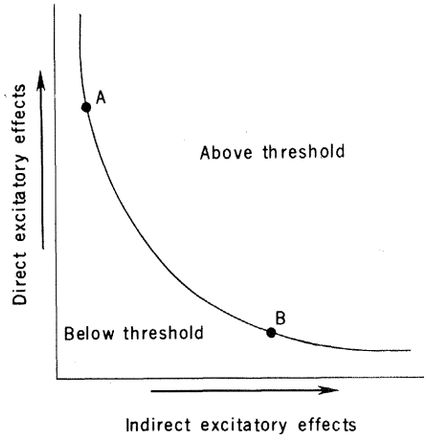

Fig. 2. A model of opiate withdrawal that is based on the assumption that opiate withdrawal signs reflect a synergistic interaction of direct and indirect excitatory phenomena. All points along the line reflect the combination of direct and indirect excitatory effects that will produce withdrawal. Points to the left of the line reflect conditions that are below withdrawal threshold, and points to the right are above threshold. Different points along the threshold line reflect changes in the ratio of direct to indirect effects. For example, at *A*, direct excitatory requirements are high because indirect excitatory effects are low; similarly, at *B*, less direct excitation is needed because of increases in indirect excitation. The present data indicate that naloxone-precipitated withdrawal occurs largely as a result of direct morphine action on excitatory nonspecific receptors and indirectly because of the prevention of morphine (and endogenous opiate) depression by stereospecific receptor blockade. In long-term addiction, withdrawal presumably results from direct excitatory morphine effects plus the indirect hyperexciting effects of a global "disequilbrium shock" triggered by sudden cessation of opiate administration.

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