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Opiate Dependence Following Acute Injections of Morphine and Naloxone: The Assessment of Various Withdrawal Signs

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RITZMANN, R. F. *Opiate dependence following acute injections of morphine and naloxone The assessment of various withdrawal signs*. PHARMAC. BIOCHEM. BEHAV **14**(4) 575–577, 1981.—The injection of high dose of naloxone 15 minutes after a single injection of morphine in mice was found to produce a jumping response which was behaviorly similar to the jumping response observed during the withdrawal from chronic morphine administration. In addition the jumping response following the acute administration of morphine-naloxone was increased by the injection of atropine and attenuated by oxotremorine These data are consistent with the reports of effect of these cholinergic drugs on the jumping response which occurred during withdrawal after chronic morphine administration. However, other symptoms associated with opiate withdrawal (hypothermia, weight loss and diarrhea) were not produced by the acute injection of morphine-naloxone. It is therefore suggested that this single injection paradigm is particular to the jumping response rather than a demonstration of the rapid development of opiate dependence.

Physical dependence Morphine Naloxone Jumping Hypothermia

IT has been shown that the injection of the morphine antagonist, naloxone either just before or 15 minutes after a single dose of morphine is capable of producing a withdrawal-like jumping response in mice [4,7]. These data were interpreted by the authors as evidence for the rapid development of physical dependence. Jacquet [4] has proposed that morphine interacts with two functionally different types of receptors: one is stereospecific and naloxone reversible; the second is neither stereospecific or naloxone sensitive. The former of these two classes of receptors has been suggested to be involved in the depressing effects of morphine and inhibiting the second class of receptor, which mediates the excitatory response to the drug. The displacement of morphine from the naloxone sensitive receptor would reverse the depressing effects of the opiate and through disinhibition allow the excitatory responses to be manifested i.e., the hyperexcitable state of withdrawal. Although this model adequately accounts for the results of several experiments measuring naloxone-induced jumping, several authors have cautioned against using a single parameter to assess opiate dependence [1]. Results from our laboratory have shown that certain symptoms of morphine withdrawal (i.e., hypothermia) can be prevented from developing by the administration of a number of peptides structurally related to Pro-Leu-Gly-NH_2 (MIF), while other symptoms, particularly jumping are unaffected by these treatments [5,8]. Way *et al.* [9] has demonstrated a cholinergic involvement in the jumping response which occurs during naloxone-induced withdrawal from chronic morphine administration. On the other hand our data, as well as others, suggest withdrawal hypothermia is mediated by dopamine systems [2, 3, 6]. To further evaluate the model proposed by Jacquet [4], we monitored mice injected with morphine and naloxone for the occurrence of a number of withdrawal signs including jumping, hypothermia, body weight loss and diarrhea. We also compared cholinergic drugs for their ability to modify the naloxone-induced jumping response following acute morphine with the reports of the effects of these drugs on the jumping response subsequent to chronic morphine administration [9].

Male Swiss Webster mice (Scientific Small Animal Farms Inc., Melrose Park, IL) weighing 25 ± 3 g (mean ± SD) were used in these experiments. Mice were housed 5–6 per cage in temperature (23 ± 1°C) and light (light 0800–1800 hr) controlled rooms and were kept in our laboratories for a minimum of 7 days prior to the initiation of experiments. Food (Purina Laboratory Chow) and water were available ad lib.

The ability of an acute IP injection of morphine (Mallinckrodt Inc., St. Louis, MO) and naloxone (Endo Laboratories, Garden City, NY) to produce various withdrawal signs was determined as described by Stevens and Klemm [7]. Mice were injected with morphine (50 mg/kg) then 15 minutes later injected with naloxone (100–150 mg/kg). Control animals received injections of either saline-naloxone, morphine-saline or saline-saline. Body temperature was determined with a tele-thermometer (Yellow Springs Ins. Co., Yellow Springs, OH) and a lubricated probe inserted 2 cm into the rectum. Measurements were made just prior to the first injection, 15, 30 and 60 minutes post injection. At the time body temperature was determined mice were also monitored for the occurrence of diarrhea. Body weight was measured just prior to

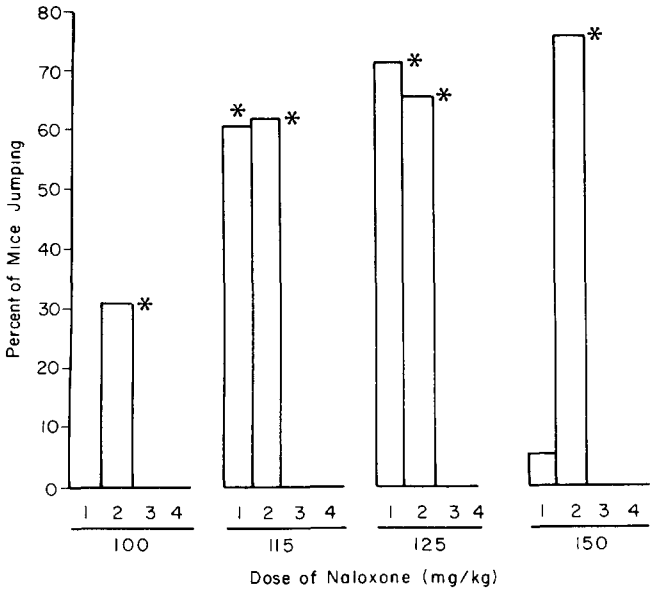

FIG 1 The effect of cholinergic drugs on morphine-naloxone induced jumping in mice. Mice were injected with morphine-naloxone (group 1), morphine-Atr-naloxone (group 2), morphine-Oxt-naloxone (group 3) and monitored for the number of mice jumping. Controls (group 4), mice injected with saline in place of any of the three injections, did not exhibit any jumping and were pooled for illustrative clarity Each group contained 15–30 mice *p=0.01 Fisher's Exact probability test compared with appropriate control group

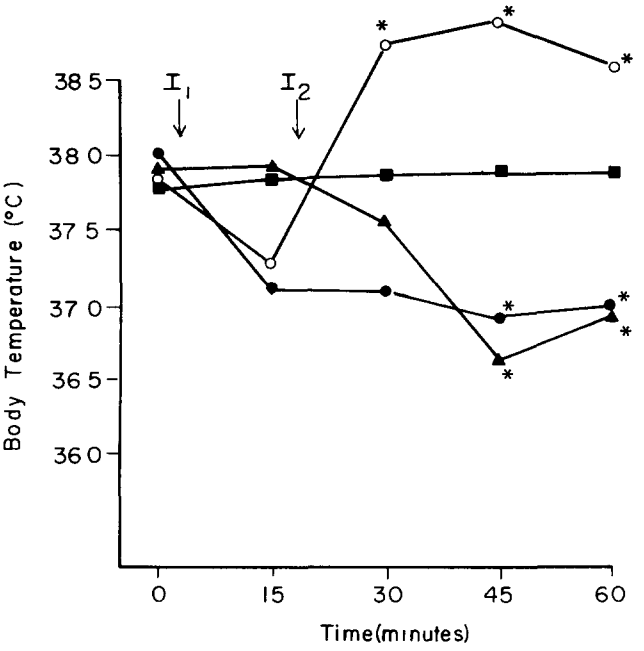

FIG. 2. The effect of the injection of morphine-naloxone on body temperature in mice. Morphine (50 mg/kg) or saline was injected (I_1) fifteen minutes later naloxone (125 mg/kg) or saline was injected (I_2) The 4 groups were· saline-naloxone (▲–▲), morphine-saline (○–○), morphine-naloxone (●–●), and saline-saline (■–■). Body temperature was recorded at various times after the injections Each group consisted of 20 mice *p>0 01 Duncan's multiple range test.

and 60 minutes after the first injection. Naloxone-induced jumping was assessed by placing the mice in plastic cylinders 21 cm in diameter and 22 cm in height immediately after the second (naloxone or saline) injection. The number of mice jumping as well as the number of jumps per mouse was recorded for a fifteen minute period. The effect of the cholinergic agonist, oxotremorine (Oxt) and antagonist atropine (Atr) on the jumping response was assessed in separate groups of mice by injecting Oxt (0.1 mg/kg), Atp (2 mg/kg) or saline 5 minutes after the first injection (morphine or saline). Ten minutes after the injection of the cholinergic compounds the animals were injected with naloxone (100–150 mg/kg) and the jumping response was measured as described above.

As previously reported [7] the injection of naloxone at doses of 115 and 125 mg/kg 15 minutes after the injection of morphine (50 mg/kg) produced a withdrawal like jumping response in mice, while higher (150 mg/kg) or lower (100 mg/kg) were ineffective (Fig. 1). Similar to the findings of Way *et al.*, [9] in chronic morphine treated mice Atr increased the effectiveness of naloxone at both the higher and lower doses. Likewise, Oxt blocked the jumping response at all doses of naloxone tested (Fig. 1). On the other hand, the acute morphine-naloxone treatment did not produce any other sign associated with opiate withdrawal. That is, none of the mice showed any loss of body weight 60 minutes after the naloxone injection ($p<0.05$), nor did any of the mice have any signs of diarrhea during the 60 minute test period. The results of the morphine-naloxone treatment on body temperature is shown in Fig. 2. Morphine produced a biphasic effect on body temperature, an initial hypothermia followed by a hyperthermic response 30 minutes post injection. Naloxone (125 mg/kg) produced a slight hypothermic response in nonmorphine treated mice. The injection of naloxone in morphine injected mice blocked the hyperthermic response but did not increase the hypothermic response as is observed in chronic morphine treated mice [2, 3, 5, 8].

These data indicate that while the concomitant injection of morphine and naloxone does produce a withdrawal like jumping response, which pharmacologically responds similar to the jumping response observed following chronic morphine administration, other signs of physical dependence are not apparent. While it might be argued that jumping is a more sensitive measurement of opiate dependence or develops before other signs of dependence this is not consistent with the reports of Blasig *et al.*, [1] that jumping is one of the last signs of dependence to develop during chronic morphine treatment. Therefore some degree of caution should be used in employing this paradigm to study morphine dependence.

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