MOTIVATIONAL INDICES OF SPONTANEOUS OPIATE WITHDRAWAL IN MICE

Papaleo F., 1° anno di corso del Dottorato in Farmacologia e Tossicologia XVII ciclo. Durata del Dottorato in anni: 3. Sede di servizio: Dipartimento di Farmacologia e Anestesiologia "Egidio Meneghetti", Largo Meneghetti 2, 35131 Padova.

Opiate addiction is a major health problem of our society. Following repeated use, abrupt opiate withdrawal results in characteristic somatic and affective symptoms, such as a flu-like state and feelings of anxiety and/or dysphoria. Withdrawal symptoms are thought to play a crucial role in driving continued drug abuse and motivational effects of opiate withdrawal have also been reported to have an important role in triggering relapse to drug use even after a long period of abstinence. Understanding of the neurobiology underlying the affective symptoms of opiate withdrawal remains thus a major goal in drug abuse research. However, despite numerous efforts have been devoted to the investigation of the neural mechanisms mediating opiate addiction, to date relatively few studies have been carried out to establish animal models able to reliably detect affective-like states present during spontaneous opiate withdrawal. The aim of the present study was thus to examine affective-like and anxiety-like behaviors in mice undergoing spontaneous opiate withdrawal. For this purpose, mixed background C57BL/6x129Sv-Ter mice were repeatedly treated with morphine and tested in the Conditioned Place Aversion (CPA) and Elevated Plus-maze (EPM) behavioral paradigms.
During the CPA experiment, initially each mouse was allowed to explore freely the entire CPA apparatus for 20 min (pre-conditioning test). The time spent in each of the two distinct CPA compartments was measured. Subsequently, during six consecutive days every 12 hours (8 to 10 a.m.; 8 to 10 p.m.) the mice were treated with vehicle (physiological saline) or increasing doses of morphine according to the following protocol: day 1: 20 mg/kg, day 2: 40 mg/kg, day 3: 60 mg/kg, day 4: 80 mg/kg, day 5: 100 mg/kg, day 6: 100 mg/kg only a.m. Moreover, on days 3 to 6, eight hours after the a.m. treatment each mouse was confined daily for 30 min in the preferred side of the CPA apparatus as determined during the pre-conditioning test. Mice were given a total of four conditioning trials during which opiate withdrawal signs such as wet dog shake, body stretch, jumping and decreased levels of locomotor activity were also quantified. Presence or absence of diarrhea during conditioning trials was also recorded. Since the morphine treatment schedule adopted here produced strong body weight loss, CPA post-conditioning tests were carried out 6 days after the cessation of drug administration when morphine-treated mice showed body weight values similar to those of control mice. During the CPA post-conditioning test, mice previously treated with morphine showed strong place aversions for the compartment of the CPA apparatus associated with the spontaneous opiate withdrawal. In contrast, control mice displayed no change in spatial preference for the two compartments of the CPA apparatus with regard to the pre-conditioning test values. These results indicate that during spontaneous morphine withdrawal the mice experienced negative affective-like states and were able to associate the motivational effects of opiate withdrawal with the environmental stimuli to which they were confined.
Separate groups of mice were also treated with saline or morphine according to the treatment schedule described above (morphine doses: 20-100 mg/kg, i.p.). Eight hours after the 60 mg/kg a.m. morphine treatment, both vehicle- and drug-treated mice were tested for 5 min in the EPM apparatus. During the EPM test, mice treated with morphine showed heightened levels of anxiety-like behaviors as compared to saline-treated mice. In particular, mice undergoing spontaneous morphine withdrawal spent less time and visited less frequently the "aversive sections" (open arms) of the EPM apparatus as compared to control mice. During the 5-min exposure to the EPM test, physical signs of morphine withdrawal were also monitored. In line with the CPA findings, the EPM results also indicate negative affective-like states in mice undergoing spontaneous opiate withdrawal. Following the EPM test, the mice were treated as mentioned above, i.e. morphine-treated mice were exposed to the 80-100 mg/kg doses of the drug whereas control mice were treated with saline. Finally, four hours after last morphine dosing (100 mg/kg), both control and drug-treated mice were injected with the opiate antagonist drug naloxone (1 mg/kg, s.c.), immediately placed in Plexiglas cylinders and physical signs of naloxone-precipitated morphine withdrawal were quantified by direct observation for 30 min. In contrast to saline-treated mice, naloxone produced the characteristic physical signs of morphine withdrawal, such as escape attempt (jumping), wet dog shake, chewing, ptosis, diarrhea and body weight loss in opiate-treated mice.
Overall, the results of this study demonstrate the possibility to obtain reliable behavioral indices of altered affective-like states in mice undergoing spontaneous morphine withdrawal. Findings suggestive of opiate

withdrawal-induced negative affective states were in fact obtained in two different behavioral models, i.e. the CPA and EPM tests. The behavioral paradigms described here may also prove very useful to examine the selective role of specific genes/proteins in opiate withdrawal-induced motivational states by using genetic mouse models.

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