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BRIEF COMMUNICATION

A Preliminary Description of Acute Physical Dependence on Morphine in the Vervet Monkey

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KRYSTAL, J. H. AND D. E. REDMOND, JR. *A preliminary description of acute physical dependence on morphine in the vervet monkey.* PHARMACOL BIOCHEM BEHAV **18**(2) 289–291, 1983.—Previous animal studies have suggested the rapid development of opiate dependency in 24 hours or less. However, the development of dependence on opioids within twenty-four hours has yet to be demonstrated in previously opiate-free human or nonhuman primate subjects. Following naloxone administration, cable-restrained monkeys which received intravenous morphine hourly for only six hours exhibited a behavioral syndrome characteristic of opioid withdrawal in this particular species. These data indicate that acute physical dependence on morphine may be induced after six hours in a primate species.

Opiate Morphine Vervet monkey Naloxone Abstinence syndrome Physical dependence

A NUMBER of studies in the rodent and dog suggest that dependence on opioids administered for 24 hours or less may be demonstrated by challenge with the opiate antagonist, naloxone [2, 3, 7, 10, 11, 13, 15, 18, 24]. However, a number of these studies employed sufficiently large doses of morphine to raise the question of relevance to human uses of opiates and selectivity of morphine effects on opioid systems [19]. One study in cats could not elicit signs of acute opioid abstinence following a single dose of morphine and naloxone challenge [22].

Drug-free human subjects with a history of opioid dependency experienced opioid abstinence symptoms when a mixture of 40 mg methadone and 4 mg naloxone was followed a week later by another dose of this mixture [16]. The 54.8 hour secondary half-life of methadone in man raises a question as to whether this constitutes "acute" dependence [23]. Other complicating factors in this experiment included the fact that previously opioid dependent subjects and an agonist-antagonist mixture rather than an agonist alone were utilized to produce acute dependence. These factors raise the possibility that the subjects were already sensitized to the effects of opioid antagonists [5].

Because of the theoretical importance of the phenomenon of acute dependence for understanding the mechanisms of tolerance and addiction and the serious ethical issues raised by an experimental study in opiate-naive humans, we have conducted studies in previously opiate-naive non-human primates to determine whether six hours of morphine administration is sufficient to demonstrate evidence of acute dependence.

METHOD

Three male vervet monkeys (*Cercopithecus aethiops sabaeus*) weighing between 3.2 and 6.4 kg were adapted to cable restraint jackets (Alice King Chatham Medical Arts Co.) and implanted under ketamine and sodium pentobarbital anesthesia (Ketalar, 50 mg ± ml, Parke-Davis and Co., Nembutal, 50 mg/ml, Abbott Laboratories, 3.4 ml) with silastic catheters (1.2 mm i.d. × 2.0 mm o.d.) into the internal jugular or cephalic vein. The silastic catheters were passed through a stainless steel restraining cable attached to a swivel chair (Alice King Chatham Medical Arts Co.) which permitted free movement within the cage. A continuous intravenous infusion (0.9% NaCl solution in water (saline) or 5% dextrose and 0.225% NaCl, with 1 unit/ml heparin (Upjohn Co.) and 0.2 mg/ml chloramphenicol (Chloromycetin Sodium Succinate, Parke-Davis, and Co.)) was maintained at a rate of 9 ml/hr at all times except during drug injections. The monkeys were caged individually.

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TABLE 1

BEHAVIORS OBSERVED DURING SALINE, MORPHINE, NALOXONE OR NALTREXONE ADMINISTRATION IN MONKEYS

	NaCl††	Naltrex‡‡	MS	Naloxone					
Nalo. Inject No.	—	—	—	1	2	3	4	5	6
No. Monkeys	9	3	3	3	3	3	3	2	2
Obs./Monkey	12	9	6	1	1	1	1	1	1
No. Obs.	108	27	18	3	3	3	3	2	2
Behaviors									
Body Shake‡	0.1±0.1	0.0±0.0	0.7± 0.7	1.0±0.6**	0.3±0.3	1.0±0.6**	0.0± 0.0	0.5± 0.5	
Cage Pick‡	0.1±0.0	0.0±0.0	0.3± 0.3	0.0±0.0	0.7± 0.7	0.0±0.0	0.0± 0.0	0.5± 0.5	
Chew‡	3.1±0.3	2.3±0.5¶	16.3±10.4**	18.0±4.7**	6.0± 1.7**	9.3±2.7**	4.5± 1.5	2.0± 0.0	
Eat†	1.4±0.6	0.0±0.0¶	10.3± 8.4	2.7±2.7	0.0± 0.0	0.0±0.0	0.0± 0.0	0.5± 0.5	
Eyes Closed*	5.1±1.1	0.0±0.0§	0.0± 0.0	0.0±0.0	0.0± 0.0	0.0±0.0	0.0± 0.0	26.0±26.0	
Freeze*	2.4±0.6	20.4±4.2§	0.0± 0.0	0.3±0.3	0.7± 0.7	0.0±0.0	0.0± 0.0	0.0± 0.0	
Lacrimat.†	0.0±0.0	0.0±0.0	3.3± 2.9**	2.7±2.7	0.0± 0.0	0.0±0.0	0.0± 0.0	0.0± 0.0	
Piloerect.†	0.0±0.0	0.0±0.0¶	1.0± 1.0	0.7±0.7	0.0± 0.0	0.0±0.0	12.0±12.0	0.0± 0.0	
Scratch‡	3.9±0.4	0.0±0.0	1.0± 1.0	0.7±0.7	0.0± 0.0	0.3±0.3	0.0± 0.0	0.5± 0.5	
Self-Groom‡	4.8±0.6	6.3±1.5	1.0± 1.0	1.7±1.2	22.3±13.0**	19.0±9.9**	26.0±25.0	13.5±13.5	
Shift‡	0.3±0.1	1.1±0.3	1.7± 1.7	3.3±1.8**	1.3± 1.3	0.7±0.7	0.0± 0.0	0.5± 0.5	
Threaten‡	0.1±0.1	0.5±0.2	0.0± 0.0	0.0±0.0	0.0± 0.0	0.0±0.0	0.0± 0.0	0.0± 0.0	
Tremor†	0.6±0.3	0.3±0.1	1.3± 0.7	1.7±0.9	0.0± 0.0	0.3±0.3	2.5± 2.5	0.0± 0.0	
Urination†	0.0±0.0	0.0±0.0	2.3± 1.9**	0.3±0.3	0.0± 0.0	0.0±0.0	0.0± 0.0	0.0± 0.0	
Vocalize†	0.1±0.0	0.7±0.3	0.0± 0.0	0.3±0.3	0.0± 0.0	0.0±0.0	0.0± 0.0	0.0± 0.0	
Vomit†	0.0±0.0	0.0±0.0	2.7± 2.7	0.0±0.0	0.0± 0.0	0.0±0.0	0.0± 0.0	0.0± 0.0	
Yawn	0.8±0.1	0.4±0.2	2.7± 2.7	6.0±1.0**	2.7± 1.5**	4.0±0.6**	0.5± 0.5	1.0± 1.0	

Behaviors were tabulated in the following manner: *present for an entire 5 second interval receives a score of 1, †scored only once for any one or more occurrences in a 5 second interval or ‡absolute frequency of defined behavior recorded during the observation period. All significance determinations are $p<0.05$ (two tailed) as determined by the appropriate Student's t-test. Except for the morphine data, all data are reported as mean value±standard error of the mean.

§Indicates consistently present during interval.

¶One count of the behavoir occurred in one monkey.

**Indicates behaviors significantly greater than those seen during both saline and naltrexone administration alone.

††Indicates comparison data from another study [11].

An initial dose of 3 mg/kg morphine sulfate was administered (Eli Lilly and Co.) via the intravenous catheter. Each successive hourly dose contained 6 mg/kg morphine. Six hours after the first dose of morphine, each monkey received 4–6 successive intravenous doses of 0.4 mg naloxone hydrochloride (Endo Laboratories Inc) at 5 minute intervals for a total of 1.6–2.4 mg naloxone. Behavioral signs and symptoms were recorded which have been found to reflect opioid withdrawal in the monkey [1, 17, 20]. In each observation session, an experienced observer sitting with a clear view of the cage recorded data using behavioral categories and methods described elsewhere [8]. Since the silastic catheter delayed delivery of drug to the vein, observations of drug effects began at the time that drug was injected into the monkey (one to two minutes after infusion into the catheter). Each observation session of drug effects began approximately at the start of drug infusion into the vein (1–2 minutes after infusion was begun into the silastic catheter). Comparison data were collected after 0.9% NaCl administration to nine monkeys for 12 sessions each by one or two observers similarly in view and at the same distance from the monkeys. Comparison data were also collected from three of these monkeys for 9 similarly observed sessions after administration of the long-acting opiate antagonist, naltrexone (22 mg/kg). Correlated and uncorrelated Student's t tests, as appropriate, were used to compare post-naloxone behaviors with those observed during morphine administration and during saline or naltrexone administration in the control group.

RESULTS

In the morphine treated monkey, naloxone precipitated an abstinence syndrome which could be distinguished from saline or morphine administration alone (Table 1). Compared with monkeys administered saline, or naltrexone without previous morphine administration, monkeys receiving naloxone following morphine administration demonstrated increases in behaviors such as chew, lacrimation, yawn, shift, and urination, and they showed a decrease in scratch and "threaten outside of cage." Although there were no statistically significant differences in the occurrence of vomiting in the three groups, the fact that vomiting was observed only after naloxone in the acute opioid dependence group suggests that this well-known sign of opiate withdrawal may have been due to naloxone administration as well.

DISCUSSION

These findings in the vervet monkey support previous work which suggests that acute physical dependence on opioids may develop after short-term administration. Symptoms of opioid abstinence were produced after relatively low

doses of morphine plus naloxone. This acute opioid abstinence syndrome was distinguished from behavior observed during a comparison study of saline or naltrexone administered alone under similar conditions.

In evaluating the evidence for acute opioid dependence, one must distinguish, if possible, between behaviors which arise as a direct effect of naloxone and behaviors which arise from precipitated opioid abstinence. Most [4, 6, 16, 21] but not all [9] studies have found little evidence for opioid withdrawal behaviors after naloxone or naltrexone alone, and behavioral changes were not seen in our comparison group of opiate-naive *C. aethiops* after large doses of naltrexone.

These preliminary data suggest the possibility that acute dependence may provide a useful and meaningful model for more chronic states. We observed many of the same behavioral signs of opioid abstinence under acute conditions as were observed after naloxone administration to monkeys which had been implanted with morphine pellets for 10 days [8]. Although withdrawal phenomena have not been identified after acute administration of opioids without naloxone challenge, short-term administration of morphine appears to serve, at least in some respects, as a useful model for more chronic states in the primate. Further work is necessary to elucidate the parameters of acute dependence, to describe the underlying biochemical mechanisms, and to determine the clinical relevance of this phenomenon.

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