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Dependence Studies of New Compounds in the Rhesus Monkey and Mouse (1991)

M.D. Aceto, E.R. Bowman, L.S. Harris and E.L. May

All the compounds except morphine·SO_4, codeine·PO_4, meperidine·HCl, bulbocapnine·HCl, NIH 10671 and buspirone·HCl were supplied by Dr. Arthur Jacobson, Laboratory of Medicinal Chemistry, NIDDK, NIH. The identities of all the compounds, except those indicated above, were unknown to us when they were originally submitted. These studies were conducted under the auspices of the Committee on Problems of Drug Dependence.

Dependence Liability Studies in Rhesus Monkeys

Substitution for Morphine (SDS) Test. Male and female rhesus monkeys (M. mulatta) weighing 2.5-7.5 kg were used, and they received 3 mg/kg, s.c., or morphine·SO_4 every 6 h. All the animals had received morphine for at least 3 months and were maximally dependent on morphine (Seevers and Deneau 1963). A minimal 2-week recuperation period was allowed between tests. At least 3 monkeys/dose were used. The assay (Aceto and co-workers, 1977 and 1978) was initiated by a subcutaneous injection of the test drug or control substances (morphine and vehicle) into animals in a group that had not received morphine for 14-15 h and showed definite signs of withdrawal. Each animal was randomly chosen to receive one of the following treatments: a) a dose of the compound under investigation; b) morphine control, 3.0 mg/kg; and c) vehicle control, 1 ml/kg. The animals were scored for suppression of withdrawal signs during a 2.5-h observation period. The observer was "blind" regarding the choice of treatments. At the end of the study, the data were grouped according to dose and drug. The mean cumulative score ± SEM was calculated and the data illustrated in figure form.

Precipitated Withdrawal (PPT-W) Test. This evaluation was done under the same conditions as described above, except that the animals were administered a test compound 2-3 h after the last dose of morphine. These animals were not in withdrawal. Naloxone·HCl (0.05 mg/kg, s.c.) served as the positive control.

Primary Physical Dependence (PPD) Study. Drug-naive monkeys were medicated with drug, using escalating dose regimens, periodically challenged with naloxone or placed in abrupt withdrawal. They were observed for overt behavioral signs during drug administration and when they were challenged with antagonist or abruptly withdrawn from the drug.

Rat Infusion Studies

The continuous infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. Rats were anesthetized after which each was fitted with a specially prepared cannula which was passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted into the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a flow-through swivel mechanism which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe which was attached to a syringe pump. The animals received 7-10 ml of solution every 24 h. Occasionally, when deemed necessary, as with cocaine, infusions were given *via* the right jugular vein.

Substitution for Morphine (SM) Test. The rats received morphine·SO_4 (50 mg/kg/24 h on the first day, 100 mg/kg/24 h on the second day, and 200 mg/kg/24 h from days 3-6). Then, a test drug was substituted for 2 days. The morphine controls received an infusion of water. The animals were observed for changes in body weight and for behavioral-withdrawal signs for 0.5 h at 6, 24, 48, 72 and/or 96 h after stopping the infusion of morphine.

Primary Pphysical Dependence (PPD) Study. The rats received test compound, as specified below, for 6 days and then, were placed in abrupt withdrawal and observed for overt behavioral signs.

Mouse Antinociception Tests

Male mice, weighing 20-30 g, were used. All drugs were dissolved in distilled water or in the vehicle indicated and injected subcutaneously (s.c.). At least three doses were tested, and 6-10 animals per dose were used. When applicable, ED50's were calculated by using computerized probit analysis.

Tail-Flick (TF) and (TF vs M) Assays. The procedure and modifications were described (D'Amour and Smith, 1941 and Dewey et al., 1970 and 1971) in the literature. Briefly, the mouse's tail was placed in a groove which contained a slit under which was located a photoelectric cell. When the heat source of noxious stimulus was turned on, the heat focused on the tail, and the animal responded by flicking its tail out of the groove. Thus, light passed through the slit and activated the photocell which, in turn, stopped the recording timer. The heat source was adjusted to produce tail flick of 2-4 s under control conditions. Mice were injected with drug or vehicle and tested 20 m later. In the assay for antagonism of the antinociceptive effect, the potential antagonists were administered 10 m before the agonist, and evaluation occurred 20 m later.

Phenylquinone Abdominal-Stretching (PPQ) Assay. The procedure was reported previously (Pearl and Harris, 1966). The mice were injected with test drugs and 10 m later received 2.0 mg/kg ip of a freshly prepared paraphenylquinone (PPQ) solution. The mice were then placed in cages in groups of two each. Ten m after the PPQ injection, the total number of stretches per group were counted over a 1-m period. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles, and extension of the forelimbs. The antinociceptive response was expressed as the percent inhibition of the PPQ-induced stretching response.

Hot-Plate (HP) Assay. The method was also reported previously (Eddy and Leimbach, 1953 and Atwell and Jacobson, 1978). The hot plate was held at 55°C. Mice were placed on the hot plate and activity was scored if the animal

jumped or licked its paws after a delay of 5 s or more, but no more than 30 s beyond the control time.

Table 1

Comparative Data (ED50, mg/kg s.c.) [95% C.L.] of Selected Standards in 4 Mouse Agonist-Antagonist Tests at MCV/VCU

Drug	Tail-Flick	Tail-Flick Antagonist	Phenylquinone	Hot-Plate
Pentazocine	15% at 10.0	18 (12-26)	1.7 (1.0-2.5)	- - - -
Cyclazocine	17% at 1.0[a]	0.03 (0.020-0.78)	0.01 (0.005-0.03)	- - - -
Nalorphine·HCl	None at 10.0	2.6 (0.7-10.0)	0.6 (0.03-1.44)	- - - -
Naloxone·HCl	None at 10.0	0.04 (0.01-0.09)	No Activity	- - - -
Naltrexone·HCl	None at 10.0	0.007 (.002-0.02)	No Activity	- - - -
Morphine·SO_4	5.8 (5.7-5.9)	Inactive	0.23 (0.20-0.25)	0.85 (0.39-1.86)
Codeine·PO_4	- - -	Inactive	- - -	6.4 (0.39-16.8)
Meperidine·HCl	- - -	Inactive	- - -	4.6 (1.8-11.7)

[a]Mice were ataxic at 3.0 and 10.0 mg/kg but there was no further increase in reaction time.

Table 2

Comparative Data (ED50 mg/kg) [95% C.L.] from the Hot Plate Assay[a]

	Hot Plate s.c./p.o.
Morphine·SO_4	0.98 (0.83-1.1) 8.3 (6.0-11.4)
Codeine·PO_4	6.8 (4.5-10.2) 13.5 (9.7-18.7)
Levorphanol Tartrate	0.2 (0.1-0.3) -
Meperidine·HCl	5.3 (4.0-7.1) -
(-)-Metazocine·HBr	0.6 (0.5-0.9) 10.6 (8.0-14.1)
Dihydromorphinone·HCl	0.19 (0.15-0.25) 0.9 (0.7-1.2)
Nalorphine·HCl	9.9 (5.7-2.1) -
Cyclazocine	1.5 (1.1-2.1) -
Pentazocine	9.3 (6.7-12.8) -
Chlorpromazine·HCl	1.1 (0.9-1.5) -

Phenobarbital, amobarbital, oxazepam, flurazepam, meprobamate and mescaline are inactive in the hot plate test.

[a]Data from Table 2 supplied by Dr. A. Jacobson

SUMMARY OF NEW DATA

Compound NIH	Chemical Name or Generic Class	MOUSE TF	MOUSE TF vs M	MOUSE PPO	MOUSE HP	RAT SM	RAT PPD	MONKEY SDS	MONKEY PPt-W	MONKEY PPD
0001	Morphine·SO_4				+					
0002	Codeine·PO_4				+					
5221	Meperidine·HCl				+	+	+			
10567	Ibogaine·HCl	+	+	+				+		+
10577	(-)-1S,2S-U50,488-MeI	+	+	+	+			+		
10578	(+)-1R,2R-U50,488-MeI	+	+	+	+			+		
10593	1-Chlorobuprenorphine	+	+	+					+	
10615	Bulbocapnine	+	+	+[a]						
10629		+	+	+				+		
10637		+	+	+				+		
10644		+	+	+				+		
10645		+	+	+						
10647	Mirfentanil	+[b,c]			+					
10650		+	+	+				+		
10651		+	+	+				+		
10655		+	+	+				+		
10656	BOZO	+	+	+				+	+	
10657		+	+	+				+		
10658		+	+	+						
10659		+	+	+						
10660		+	+	+						
10663	Nalmefene glucuronide	+[b]		+[a]						
10665		+	+	+				+		
10666		+	+	+				+		
10667		+	+	+					+	
10671		+	+	+						
10672		+[b]		+	+	+	+	+		+

SUMMARY OF NEW DATA (cont.)

Compound NIH	Chemical Name or Generic Class	MOUSE TF	MOUSE TF vs M	MOUSE PPQ	MOUSE HP	RAT SM PPD	MONKEY SDS	MONKEY PPt-W	MONKEY PPD
10673	6,7-Benzomorphan	+	+	+			+		
10674	6,7-Benzomorphan	+	+	+			+		
10675	6,7-Benzomorphan	+	+	+					
10676	Azabicyclononanone	+	+	+					
10677	Diazaadamantane	+	+	+	+				
10679	LAAM, Acetylmethadol	+	+	+			+		
10680	Ketobemidone	+	+	+			+		
10681	Ketobemidone	+	+	+			+		
10682	Benzoylecgonine	+	+	+			+		
10685	Morphine	+[b]	+	+	+		+		+
10687	Buspirone	+	+	+[a]	+				
10700	Piperazine	+	+	+	+				
	N-Phenyl-N-cyclohexyl-3,4-Dehydropiperidine								
10713	Diazabicyclononanone	+	+	+	+				
10714	Diazabicyclononanone	+	+	+	+				
10715	Diazabicyclononanone	+	+	+	+				

[a]Naloxone vs ED80 in PPQ test, [b]Naloxone prior to ED80 in TF test, [c]Naloxone after ED80 in TF test

NIH 0001 Morphine·SO_4

MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF -
2) TF vs. M -
3) PPQ -
4) HP - 1) 0.85 (0.39 - 1.86)
   2) 0.72 (0.34 - 1.49)
   3) 0.98 (0.83 - 1.1)[a]

[a]Reported by Dr. Jacobson

NIH 0002 Codeine·PO_4

MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF -
2) TF vs. M -
3) PPQ -
4) HP - 1) 6.4 (2.4 - 16.8)
   2) 6.8 (4.5 - 10.2)[a]

[a]Reported by Dr. Jacobson

NIH 5221 Meperidine·HCl

MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF -
2) TF vs. M -
3) PPQ -
4) HP - 1) 4.6 (1.8 - 11.7)
   2) 5.3 (4.0 - 7.1)[a]

[a]Reported by Dr. Jacobson

NIH 10567 Ibogaine·HCl

MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 9.7 ((2.8 - 34.0)[a]

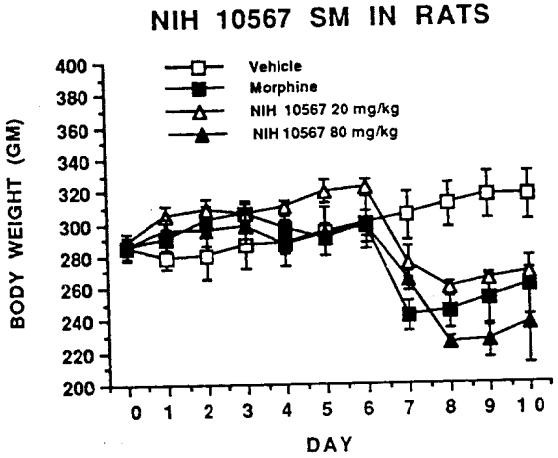
[a]Vehicle - 2% Tween 80 in water

NIH 10567 Ibogaine·HCl (cont.)

Rat Infusion

A. (SM)

As shown in the fig. (designated NIH 10567 SM) and table, NIH 10567 did not substitute for morphine at a dose of 20 mg/kg/day. However, at the high dose, namely 80 mg/kg, some suppression of behavioral withdrawal signs (see table) was noted on day 6 (24-h withdrawal). However, body-weight loss was actually greater than that of the withdrawn morphine-dependent rats (see fig.). In any case, suppression of body-weight loss is considered the most important criterion for substitution for morphine in this assay. These results may simply indicate a non-specific effect on behavior. Additional studies are suggested.



B. (PPD)

As shown in the accompanying fig.(designated NIH 10567 PPD) and table, ibogaine did not produce a significant degree of physical dependence at the dose regimen tested.

Table (NIH 10567) Primary Physical Dependence (PPD) and Substitution for Morphine (SM) Studies with Ibogaine in Continuously-Infused Rats

Treatment	Hour in Withdrawal					
	2	6	24	48	72	96
	Mean Number of Withdrawal Signs[a,b]					
1. Vehicle + Vehicle Controls[c]	0	0.3	0.3	1.0	0	0.5
2. Morphine + Vehicle Controls[d]	0	0.8	11.3[b]	16.3[b]	11.5[i]	7.5[i]
3. Morphine + NIH 10567[e] (low dose - SM)	0	0	16.5[b,j]	14.0[b,j]	10.0[b,j]	9.8[b,j]
4. Morphine + NIH 10567[f] (high dose - SM)	0	0.3	1.6[i,k]	6.8	8.3[b,j]	17.0[i]
5. NIH 10567 + Vehicle[g] (low dose - PPD)	0.3	2.3	3.5	1.8	3.8[i]	3.0[i]
6. NIH 10567 + Vehicle[h] (high dose - PPD)	0.3	1.8[i]	5.0	4.3	4.0[i]	2.5[i]

[a]Hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing and chewing; [b]One-tailed test (Mann-Whitney U-test), p = 0.05 or less compared to vehicle controls; [c]Vehicle was Tween 80 + water. Days 1-10; 8 ml/24 h; N = 4; [d]Morphine·SO_4 50 mg/kg on day 1; 100 mg/kg on day 2; 200 mg/kg on days 3-6; N = 4 on day 7, 3 on day 8 and 2 on day 10; [e]Vehicle on days 7-10; [f]Morphine·SO_4 as