

Dependence Studies of New Compounds in the Rhesus Monkey and Mouse (1990)

Mario D. Aceto, Edward R. Bowman, Louis S. Harris, and Everette L. May

All the compounds except cocaine, norcocaine, methaqualone, and the N-butyrophenone prodine-like compounds (NIH 10639, 10640, and 10641) were supplied by Dr. Arthur Jacobson, Laboratory of Medicinal Chemistry, NIADDC, 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.

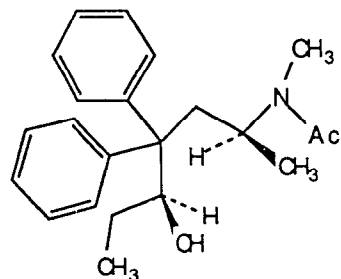
For the most part, the procedures described by Seevers and his colleagues (1936, 1963) and Deneau (1956) regarding the facilities and training of the monkeys were used and a brief description follows. The monkeys were injected with 3.0 mg/kg s.c. of morphine sulfate every 6 hr for at least 90 days before being used. This dose regimen was reported by Seevers and Deneau (1963) to produce maximal physical dependence.

Modified procedures for the precipitated withdrawal (PPt-W) and single-dose suppression (SDS) tests were reported by Aceto and co-workers (1977 and 1978). The PPt-W test was initiated by the injection of a test drug 2 1/2 hr after an injection of morphine and the animals were observed for signs of withdrawal. The SDS test was started approximately 15 hr after the last dose of morphine at which time the animals were showing withdrawal signs. The onset and duration of action of the test drug were noted. In both tests, a vehicle control and an appropriate positive control (naloxone hydrochloride, 0.05 mg/kg or morphine sulfate, 3.0 mg/kg) along with 2 or 3 different treatments (doses) of a test compound were randomly allocated to the 4 or 5 monkeys of a group. Usually, 3 or 4 groups per compound were used. All drugs were given subcutaneously (1 ml/kg) and the vehicle was water except where indicated. The observer was "blind" with regard to the treatment given. A minimal 2-week washout and recuperation period between tests was allowed.

Three mouse tests were used in our laboratory to provide a preliminary estimate of the potency and profile of activity of each test compound. The tests were the tail-flick agonist (TF) and the morphine antagonist (TF vs M) tests and the phenylquinone (PPQ) test (Dewey et al., 1970; Dewey and Harris, 1971). Reference-standard data for these tests are shown in Table 1. In addition, Dr. Jacobson occasionally provided us with estimated starting doses. These doses were based on results obtained from the mouse-hot plate assay (HP) (Eddy and Leimbach, 1953; Jacobson and May, 1965; Atwell and Jacobson, 1978). Reference data for this test are shown in Table 2.

SUMMARY OF COMPOUNDS TESTED

Compound NIH	Chemical Name or Generic Class	MOUSE		HP	MONKEY	
		TF	TF vs M		SDS	PPt-W
7589A	6,7-Benzomorphan	+	+	-	+	-
8209	6,7-Benzomorphan	+	+	-	+	-
8211	Cocaine	.	.	+	.	.
10365	4,5 α -Epoxyomorphan (Nalmephe) ^a	+	+	+	+	+
10520	N-Allyletonitazine bromide	+	+	+	+	+
10521	N-Ethyletonitazine iodide	+	+	+	+	+
10574	4,5 α -Epoxyomorphan	+	b	-	+	+
10575	4,5 α -Epoxyomorphan	+	+	-	+	+
10588	Norbinaltorphimine	+	+	-	+	+
10605	Naloxone 3,14-diacetate	+	+	-	+	+
10606	Naloxone 3,14-dipropionate	+	+	-	+	+
10607	Naloxone ethoxycarbonylhydrozone	+	+	-	+	+
10618	Benzeneacetamide	+	+	-	+	+
10619	Benzeneacetamide	+	+	-	+	+
10620	(+)-Thevone	+	+	-	+	+
10621, 10632	(+)-19-Propylthevinol	+	+	-	+	+
10623	4,5 α -Epoxyomorphan	+	+	-	+	+
10626	6,7-Benzomorphan	+	+	-	+	+
10627	6,7-Benzomorphan	+	+	-	+	+
10628	1,3-Diphenylguanidine	+	+	-	+	+
10630	14-Aminodihydromorphinone	+	+	-	+	+
10631	(-)-Thevone	+	+	-	+	+
10633	Furanindole	+	+	-	+	+



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

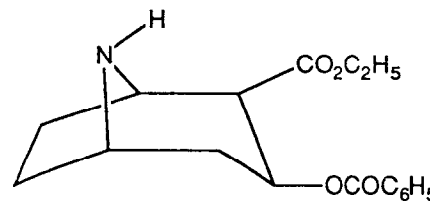
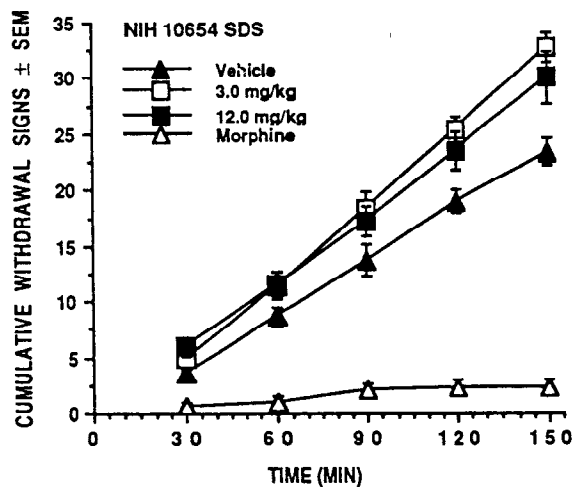
- 1) TF - 19% at 1.0, 26% at 10.0 and 7% at 30.0^{a,b}
- 2) TF vs. M - 0% at 1.0, 10.0 and 26% at 30.0^{a,b}
- 3) PPQ - Inactive at 1.0 and 10.0, 29% at 30.0^{a,b}

^aVehicle: Tween 80 + H₂O and warming

^bVehicle inactive in all tests

MONKEY DATA
SDS

NIH 10654 did not substitute for morphine at 3 or 12 mg/kg. The drug may have exacerbated withdrawal. However, the effect was not dose related. Also, vehicle control values were low magnifying possible drug effects.



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

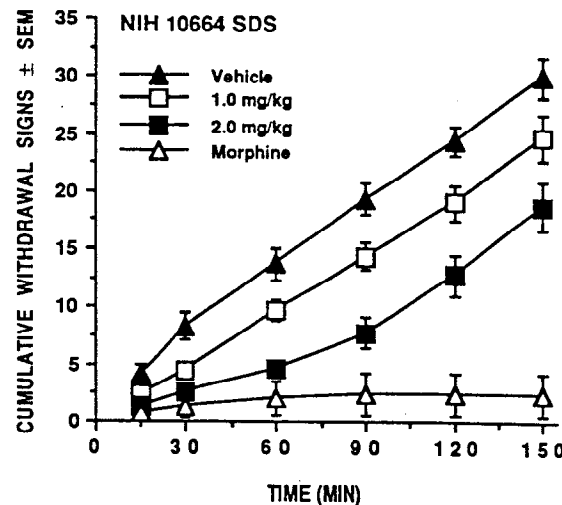
- 1) TF - Inactive at 1.0, 10.0 and 30.0
- 2) TF vs M - Inactive at 0.1, 1.0 and 10.0
- 3) PPQ - 1.65 (0.74 - 3.69)

Vehicle: Phosphoric acid and H₂O

MONKEY DATA
SDS

Kosten (1989) reported that cocaine attenuated opiate withdrawal in humans and rats. Recently we also demonstrated in our laboratory that cocaine attenuated morphine also demonstrated withdrawal in rhesus monkeys. Regarding possible mechanisms, the role of cocaine metabolites has been essentially ignored. We postulated that norcocaine, a metabolite of cocaine with central nervous system effects (Jones, 1984), played a significant role.

When norcocaine was given, it initially produced behavioral excitement which was not unlike that seen after cocaine. However, this effect dissipated in about 15-30 min after which the animal appeared normal.



Norcocaine significantly and promptly attenuated, in a dose-dependent manner, the total number of withdrawal signs (see fig.). The action peaked at 90 min and waned during the rest of the testing period. The suppressive properties of norcocaine on individual withdrawal signs is shown in the table. It is obvious that norcocaine diminished the incidence of all the individual withdrawal signs except restlessness. It should be emphasized that norcocaine did not behave like a typical mu agonist in morphine-dependent monkeys (see results in the table).

Norcocaine appeared to have a biphasic effect regarding certain signs. At the low dose it was more effective in suppressing signs designated wet-dog shakes and retching than others namely, rigid abdominal muscles and vocalizes when abdomen palpated. At the high dose, the opposite was apparent for the signs retching whose incidence increased, and vocalizes when abdomen palpated or rigid abdominal muscles whose incidence decreased. We did not attempt to administer higher doses because severe tremors suggestive of impending convulsions during the initial excitement or rausch phase were observed.

Summary

Norcocaine dose-dependently attenuates abrupt morphine withdrawal in rhesus monkeys. These results suggest a possible role for this metabolite in the interaction of cocaine with the opioid system.

TABLE 1

Comparison of the suppressive properties of norcocaine, morphine and vehicle at 90 min in withdrawn morphine-dependent monkeys

Treatment	Norcocaine	Vehicle	Morphine
Dose mg/kg i.v.	2.0	1.0	3.0
No. of Animals	4	3	3
<i>Withdrawal signs</i>			
Lying on side or abdomen	1	2	-
Fighting	1	-	-
Avoids contact	-	-	1
Vocalizes	-	-	-
Restless	7	9	3
Drowsy	-	-	-
Tremors	3	-	-
Wet-dog shakes	4	4	10
Retching	8	4	10
Vomiting	-	-	-
Vocalizes when abdomen palpated	4	12	12
Abdominal muscles (rigid)	3	1	12
Total of Signs	31	41	55
Calculated P value	a,b	a,b	a

^aSignificantly different from vehicle control ($p < 0.05$, Mann-Whitney U Test).

^bSignificantly different from morphine control ($p < 0.05$, Mann-Whitney U Test).

ACKNOWLEDGEMENTS

This study was supported by a contract (#271-87-8116) from the National Institute on Drug Abuse, Dr. Gerialin Lin, Contract Officer. We also acknowledge the expert assistance of Susan M. Tucker and Christopher C. Cull. Special thanks to Laura Johnson for her help in the preparation of this manuscript using the Macintosh II.

REFERENCES

Aceto, M.D., Flora, R.E. and Harris, L.S. The effects of naloxone and nalorphine during the development of morphine dependence in rhesus monkeys. *Pharmacol*, 15:1-9 1977.

Aceto, M.D., Flora, R.E. and Harris, L.S. Caffeine elicited withdrawal signs in morphine-dependent rhesus monkeys. *Eur J Pharmacol*, 50:203-207, 1978.

Atwell, L. and Jacobson, A.E. The search for less harmful analgesics. *Lab Animal*, 7:42-47, 1978.

Deneau, G.A. An analysis of factors influencing the development of physical dependence to narcotic analgesics in the rhesus monkey with methods for predicting physical dependence liability in man. Doctoral Dissertation, University of Michigan, 1956.

Dewey, W.L., Harris, L.S., Howes, J.F. and Nuite, J.A. The effects of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. *J Pharmacol Exp Ther*, 175:435-552, 1970.

Dewey, W.L. and Harris, L.S. Antinociceptive activity of the narcotic antagonists analogues and antagonistic activity of narcotic analgesics in rodents. *J Pharmacol Exp Ther*, 179:652-659, 1971.

Eddy, N. B. and Leimbach, D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *J Pharmacol Exp Ther*, 107:385-393, 1953.

Jacobson, A.E. and May, E.L. Structures related to morphine. XXI. 2'-Substituted benzomorphans. *J Med Chem*, 8:563-566, 1965.

Seevers, M.H. Opiate addiction in the monkey. I. Methods of study. *J Pharmacol Exp Ther*, 56:147-156, 1936.

Seevers, M.H. and Deneau, G.A. Physiological aspects of tolerance and physical dependence. In: Root, W.S. and Hofman, F.G., eds. *Physiological Pharmacology*, Vol. I. New York: Academic Press, 1963. pp. 565-570.

AFFILIATION

Department of Pharmacology and Toxicology, Medical College of Virginia Commonwealth University, Richmond, VA 23298-0613