AMP inhibition and the loss of cooperative interaction among AMP-binding sites.

The inactivation of Fru-P_2ase by trypsin has been previously reported[13,14]. In this study, we found that the immobilized Fru-P_2ase, as compared with the native enzyme, was much more resistant to inactivation by trypsin (fig. 2). This increased resistance is probably caused by steric hindrance of the approach of trypsin to the critical site of the immobilized Fru-P_2ase.

The native and the immobilized enzymes were inhibited by Zn^{2+} to about the same extent. As previously observed for free Fru-P_2ase[15], the ability of EDTA or other chelators to reverse the Zn^{2+} inhibition of the immobilized enzyme activity decreased greatly if chelators were added to the enzyme after substrate, especially when AMP was also present (data not shown).

1 This work was supported by grant RR-8006 from the General Research Branch, Division of Research Resources, NIH (USA).

2 Gomori, G., J. biol. Chem. *148* (1943) 139.
3 Falb, R.D., Lynn, J., and Shapira, J., Experientia *28* (1973) 958.
4 Han, P.F., and Johnson, J., in: Methods in Enzymology, p. 334. Ed. W.A. Wood. Academic Press, New York 1982.
5 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
6 Tashima, Y., and Yoshimura, N., J. Biochem. *152* (1975) 1161.
7 Chung, A.E., Archs Biochem. Biophys. *152* (1972) 125.
8 Kawakita, N., and Yamazaki, M., Archs Biochem. Biophys. *204* (1980) 326.
9 Axen, R., Porath, J., and Ernback, S., Nature *214* (1967) 1302.
10 Chibata, I., Immobilized Enzymes. Kodansha Ltd, Tokyo 1978.
11 Hubert, E., Villanueva, J., Gonzales, A.M., and Marcus, F., Archs Biochem. Biophys. *138* (1970) 590.
12 Taketa, K., and Pogell, B.M., J. biol. Chem. *240* (1965) 651.
13 Han, P.F., Murthy, V.V., and Johnson, J., Archs Biochem. Biophys. *173* (1976) 293.
14 Han, P.F., Han, G.Y., Hayes, R.L., Moore, C.L., and Johnson, J., Experientia *39* (1983) 1305.
15 Han, P.F., Han, G.Y., McBay, H.C., and Johnson, J., Biochem. biophys. Res. Commun. *93* (1980) 558.

0014-4754/85/091149-03$1.50 + 0.20/0

Spontaneous morphine withdrawal from the rat spinal cord[1]

D.C. Marshall[2] and J.J. Buccafusco[3]

Department of Pharmacology and Toxicology, Department of Psychiatry, Medical College of Georgia, Augusta (Georgia 30912, USA) and Medical Research Service, Veterans Administration Medical Center, Augusta (Georgia 30904, USA), 30 November 1984

Summary. A characteristic and reproducible sign of narcotic withdrawal is the naloxone induced increase in arterial pressure. In morphine-dependent rats allowed to undergo spontaneous withdrawal (6–24 h) and then transected at the spinal C-1 level, arterial pressure was maintained at a significantly higher level than either spinal-transected nondependent controls or morphine-dependent, spinal-transected rats pithed from C-1 to L-4. These findings indicate that the morphine-dependent spinal cord, independent of supraspinal influences, is able to exhibit an autonomic component of spontaneous withdrawal.

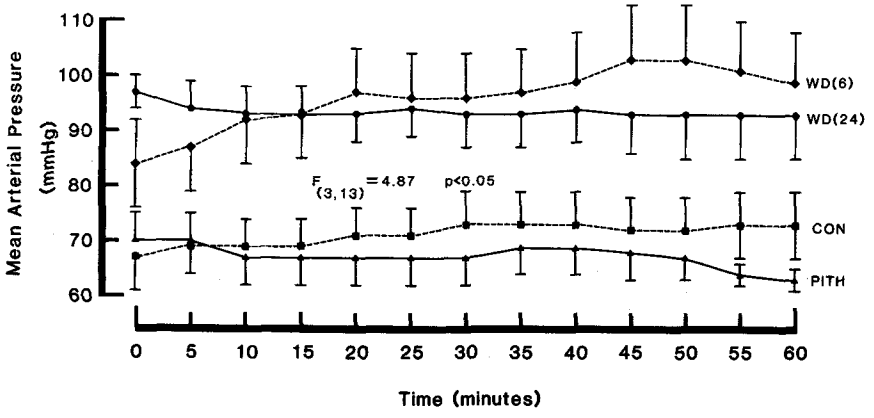
Key words. Morphine withdrawal; spinal cord; naloxone; blood pressure.

Recent studies in this[4-7] and other laboratories[8] have indicated that the spinal cord plays an important role in the expression of several signs of narcotic abstinence. As an estimate of both the degree of physical dependence and the intensity of withdrawal we have measured the post withdrawal increase in mean arterial pressure (MAP)[9-12] as well as other behavioral signs in the intact, abstinent rat. In morphine-dependent rats transected at the spinal C-1 level, systemic or local intrathecal injection of naloxone elicits a profound and long-lasting increase in MAP which can be abolished by systemic injection of autonomic blocking agents or by spinal pithing[6,7]. The dependent spinal cord, isolated from supraspinal control, should provide a relatively simplistic model for studying the mechanisms of dependence and withdrawal compared with the higher CNS. However, one important criterion for spinal mediated withdrawal has not yet been fulfilled, that is, whether the spinal cord can undergo spontaneous withdrawal. The purpose of this study, therefore, was to determine whether the increase in MAP observed in spinal-transected, morphine-dependent rats following naloxone also could be observed when the animal underwent abrupt morphine discontinuation.

Materials and methods. Male, Wistar rats (280–350 g) were anesthetized with methohexital and an aortic catheter was permanently implanted, and exteriorized to a cannula swivel mounted above the home cage to permit continuous morphine infusion or measurement of arterial pressure[9]. On the following day, morphine sulfate was infused through the catheter at a rate of 0.33 ml/h, to deliver a total dose of 35 mg/kg/day. The concentration was adjusted each morning for the next 4 days to deliver 50, 75, 100 and 100 mg/kg/day, respectively. This schedule was previously determined to induce physical dependence[11,12]. Morphine-dependent or saline-infused control rats (nondependent) were anesthetized with halothane, artificially respired and the spinal cord transected at the C-1 level. Halothane was discontinued and 30 min allowed for recovery prior to any measurements. In some animals the spinal cord was pithed with a 14 gauge trochar from C-1 to L-4.

Results. Dependent unanesthetized, freely-moving rats were deprived of morphine for 6 or 24 h (the time at which withdrawal signs first begin, and the time at which they are maximally expressed, respectively)[9]. MAP was elevated in the abstinent group compared with nondependent controls (table 1), although this was significant only in the 24-h abstinent group. Following spinal transection MAP again was higher in abstinent groups although this was not apparent in dependent, spinal pithed rats. Continuous measurement of blood pressure for 1 h revealed that MAP remained elevated throughout the 60-min observation period, in fact, the 6-h abstinent group began with a slightly lower starting MAP but eventually reached the 24-h dependent level (fig.). Again nondependent controls and dependent, spinal pithed rats maintained a lower MAP.

At the completion of the 1-h measurement period in the abstinent rats MAP remained elevated with respect to the nondependent controls; with respect to abstinent, spinal pithed animals; and, with respect to dependent rats which had not been deprived of morphine prior to C-1 spinal transection (table 2). At this



Mean arterial pressure following spinal cord (C-1) transection. CON = nondependent control rats; WD (6) = dependent rats deprived of morphine for 6 h prior to spinal transection; WD (24) = dependent rats deprived of morphine for 24 h prior to spinal transection; PITH = dependent rats deprived of morphine for 6 h prior to spinal transection and pithed from C-1 to L-4. Time 0 refers to 30 min after spinal transection. Each value represents the mean ± SEM for 4-6 animals. F = f statistic for the between treatments components by ANOVA for repeated measures.

point all five groups were injected with naloxone, 0.5 mg/kg i.a. Nondependent controls and dependent, pithed rats showed little or no response to naloxone. Dependent, non-abstinent rats responded with an immediate and marked increase in MAP of 74 mm Hg above preinjection levels which declined to 62 mm Hg above preinjection levels within 15 min (table 2). For the 6-h abstinent animals, the initial response to naloxone was similar to non-abstinent rats, however, the response declined more rapidly over the next 15 min. For the 24-h abstinent animals the pressor response to naloxone was further reduced. This, the longer the spontaneous period of withdrawal, the weaker the naloxone-precipitated response.

Discussion. Our earlier studies indicated that the spinal cord plays a role in the expression of several behavioral and autonomic signs of naloxone-precipitated morphine withdrawal in dependent rats[4-7]. The present study supports this conclusion for one autonomic withdrawal sign, the increase in MAP. It also is apparent that the increase in MAP observed to be associated with spontaneous morphine withdrawal[9] can be preserved after C-1 spinal section. The elevated MAP in these spinal-transected, abstinent animals is of spinal sympathetic origin, since it is abolished by prior destruction of the spinal cord. The failure of naloxone to generate a sustained pressor response in abstinent rats also is compatible with the concept that the spinal cord has undergone some degree of withdrawal.

Neuronal pathways arising within the pontine locus coeruleus have been suggested to contribute to the expression of enhanced sympathetic activity during naloxone precipitated withdrawal[13-15]. However, isolated preparation of this nucleus failed to exhibit an enhanced neuronal activity in the abstinent state[16]. Our results provide direct evidence that intraspinal opiate mediated circuits participate in both precipitated and spontaneous abstinence. Thus the spinal cord may provide a more simplified model for the study of the neuronal mechanisms underlying dependence and withdrawal phenomena.

Table 1. The level of mean arterial pressure before, and 6 or 24 h after morphine discontinuation in dependent, spinal-transected rats

	Prior to anesthesia	Post-transected control level	N
Nondependent control	99 ± 4	67 ± 6	4
After 6 h abstinence[a]	106 ± 3	83 ± 8	5
After 24 h abstinence	120 ± 4*	97 ± 3*	5
After 6 h abstinence and then spinal pith[b]	106 ± 6	70 ± 5	3

[a] Morphine was discontinued for 6 or 24 h in dependent rats. After recording blood pressure rats were anesthetized and the spinal cord transected at C-1. Following a 30-min stabilization period blood pressure was recorded for an additional 1 h. [b] After spinal transection the cord was pithed from C-1 to L-4. * Significantly different from nondependent control values ($p < 0.05$, by Student's t-test). Each value represents the mean ± SEM.

Table 2. The increase in mean arterial pressure (MAP) in dependent, spinal-transected rats produced by injection of naloxone 0, 6 or 24 h after morphine discontinuation

	Pre-naloxone N	Pre-naloxone Resting level	Change in MAP (mm Hg) 5 min post-naloxone	Change in MAP (mm Hg) 15 min post-naloxone
Nondependent control	4	73 ± 6	7 ± 2	−2 ± 1
Dependent, non-abstinent[a]	6	67 ± 7	75 ± 2[c]	64 ± 2
After 6 h abstinence[a]	5	99 ± 9[c,d]	80 ± 13[c]	44 ± 17[c]
After 24 h abstinence[a]	5	93 ± 7[c,d]	47 ± 7[c,d]	28 ± 6[c,d]
After 6 h abstinence and then spinal pith[b]	3	63 ± 2	11 ± 2	−2 ± 1

[a] Morphine was discontinued for 0, 6 or 24 h prior to spinal cord transection at C-1. After 1.5 h naloxone (0.5 mg/kg, i.a.) was injected and blood pressure recorded for 15 min. [b] Following spinal transection the cord was pithed from C-1 to L-4. [c] Significantly different from nondependent control values ($p < 0.05$). [d] Significantly different from dependent, non-abstinence values. Each value represents the mean ± SEM.

1 This study was supported by the Medical Research Service of the Veterans Administration. A preliminary report of aspects of this work appeared in Soc. Neurosci. Abs. *10* (1984) 1113.
2 Present address: Berlex Laboratories, Dept. Pharmacology, 110 East Hanover Ave., Cedar Knolls, NJ 07927, USA.
3 Reprint requests to: J. Buccafusco, Dept. Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912, USA.
4 Marshall, D.C., and Buccafusco, J.J., Pharmacologist *26* (1984) 197.
5 Turner, R.M., Marshall, D.C., and Buccafusco, J.J., Pharmacologist *26* (1984) 197.
6 Marshall, D.C., and Buccafusco, J.J., Soc. Neurosci. Abs. *10* (1984) 930.
7 Marshall, D.C., and Buccafusco, J.J., Brain Res. *329* (1985) 131.
8 Delander, G.E., and Takemori, A.E., Eur. J. Pharmac. *94* (1983) 35.
9 Buccafusco, J.J., Pharmac. Biochem. Behav. *18* (1983) 209.
10 Buccafusco, J.J., Marshall, D.C., and Turner, R.M., Life Sci. *35* (1984) 1401.
11 Marshall, D.C., and Buccafusco, J.J., Proc. Soc. exp. Biol. Med. *176* (1984) 216.
12 Marshall, D.C., and Buccafusco, J.J., Drug Devl Res. *5* (1985) 271.
13 Gold, M.S., Redmond, D.E., and Kleber, H.D., Am. J. Psychiat. *136* (1979) 100.
14 Crawley, J.N., Laverty, R., and Roth, R.H., Eur. J. Pharmac. *57* (1979) 247.
15 Redmond, D.E., and Huang, Y.H., J. clin. Psychiat. *43* (1982).
16 Andrade, R., Vandermaelen, C.P., and Aghajanian, G.K., Eur. J. Pharmac. *91* (1983) 161.

0014-4754/85/091151-02$1.50 + 0.20/0