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SHORT COMMUNICATIONS

Brain microsomal protein kinase in the chronically morphinized rat

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IT HAS BEEN reported that in the rat, the activities of a number of brain microsomal enzymes are characteristically affected by chronic treatment with morphine or other opioid analgesics. Such enzymes include an ATPase,[1] acetyl choline esterase[2] and a peptidase.[3] In the course of studies on the rapid induction of tolerance to and dependence on morphine in the rat, we have observed that brain microsomal protein kinase activity is also markedly affected by such treatment.

Tolerance and dependence were induced by an adaptation of the continuous infusion technique of Cox, Ginsberg and Osman.[4] Morphine hydrochloride in 0·9% NaCl solution was infused into the right external jugular vein of the rat (Wistar albino, male and female, 250 ± 30 g) at a rate of 1 ml/hr for 8 hr daily for 4 days. The rate of dosage was increased daily, the rates for each day being 5, 15, 30 and 60 mg morphine/kg/hr respectively. The degree of analgesia was measured hourly during infusions, and the analgesic index calculated as described by Cox, Ginsberg and Osman.[4] Due to the high dose levels employed the analgesic index remained maximal (1·00) throughout the second, third and fourth infusions. An estimate of the degree of tolerance attained was made by infusing a group of six animals on the fifth day at the starting dose rate of 5 mg/kg/hr. The mean peak analgesic index obtained was 0·15 ± 0·03 (S.D.) compared with 0·91 ± 0·08 obtained during the first infusion.

This 4-day treatment was sufficient to produce a clearly defined abstinence syndrome. This was precipitated by intraperitoneal injection of nalorphine hydrobromide (15 mg nalorphine/kg) 30 min after the termination of the final infusion and was characterized by profuse urination and defaecation and by the almost complete abolition of spontaneous mobility. This response to nalorphine was not observed in animals that had been infused with saline for 4 days, in saline-infused animals injected with morphine (60 mg/kg) 30 min before nalorphine, or in previously untreated animals.

As well as producing the abstinence syndrome, preliminary experiments demonstrated that this 4-day treatment produced in preparations of cerebral cortex obtained from the treated animals biochemical changes that appear to be characteristic of morphine tolerance. Thus it was found that the susceptibility to depression by morphine of K^+-stimulated respiration in isolated slices of cerebral cortex,[5] and the specific activity of microsomal Mg^{2+}-dependent ATPase in preparations obtained from chronically morphinized rats were both significantly less than in preparations from saline-infused or untreated controls.

For the determination of protein kinase activity, animals were divided into four groups. Control and acutely treated animals were not subjected to infusions, and received 1 ml of saline and 1 ml of morphine solution (60 mg/kg i.p.) respectively, 30 min before killing. Abstinent and chronically treated animals received morphine infusions for four days, as described, and received 1 ml of saline and 1 ml of morphine solution (60 mg/kg i.p.) respectively, 30 min before killing, 18 hr after the termination of the final infusion. The rats were stunned by a blow to the back of the neck and killed by exsanguination. The whole brain was removed and homogenized in seven vols 0·32 M-sucrose. The homogenates were centrifuged twice at 12,000 *g* for 20 min, the pellets being discarded. The supernatant was then centrifuged at 90,000 *g* for 90 min and the microsomal pellet thus obtained was resuspended in 1·5 ml of 0·32 M-sucrose. The protein content of the suspensions was determined by the method of Lowry *et al.*,[6] and was not found to vary significantly from group to group. Protein kinase activity in the presence and in the absence of 10 μM 3′,5′-adenosine monophosphate was determined by the method of Weller and Rodnight.[7]

Owing to the lengthy assay procedure, the activities of the preparations from untreated, acutely dosed and abstinent animals were determined at the same time, whereas the activity of the chronic preparation had to be determined in a separate experiment. The results are presented in Table 1.

It is apparent that in the acutely dosed animals protein kinase activity in the absence of cyclic AMP is not changed relative to that of the untreated animals whereas it is depressed in chronically morphinized rats and elevated in the abstinent animals. A similar pattern is seen where protein kinase activity was determined in the presence of cyclic AMP. This compound stimulates the activity in all preparations, and the percentage stimulation does not vary significantly from group to group.

The variation in the activity of this enzyme under different conditions of morphine treatment resembles the effect found for microsomal ATPase,[1] and complements the effects reported for other

TABLE 1. EFFECT OF MORPHINE TREATMENT ON BRAIN MICROSOMAL PROTEIN KINASE ACTIVITY

Treatment	Specific activiy of protein kinase + cAMP	− cAMP
Control (4)	198 ± 23	162 ± 30
Acute (4)	208 ± 31	156 ± 12
Chronic (4)	137 ± 12†	98 ± 5†
Abstinent (4)	278 ± 31*	218 ± 26*

Treatment as defined in text. Number of animals shown in parenthesis. Activities expressed as p moles ^{32} P incorporated into phosphoryl serine/mg protein in 2 min. Mean ± S.D.

* Significantly different from control $P < 0{\cdot}05$.

† Significantly different from control $P < 0{\cdot}01$.

microsomal enzymes.[2,3]. At present there is insufficient evidence to determine whether these various effects are independent or related phenomena and it would be premature to speculate as to the relationship between these changes in enzyme activity and the development of tolerance to morphine. However, it has been postulated[7] that the protein kinase examined in this work is involved in the repolarization of electrically excited tissue. It therefore seems possible that the elevated activity of this enzyme found in the abstinent animals might contribute directly to the signs of the abstinence syndrome.

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