Brain adenyl cyclase in methadone treatment of morphine dependency

The current concern over the non-medical use of narcotic analgesics together with our limited knowledge of the precise mode of action of this clinically useful class of drugs has stimulated considerable research into the neurochemical basis of the action of various narcotics. Recently, Chou, Ho & Loh (1971) found that whereas morphine failed to alter cerebral cortex phosphodiesterase, the activity of adenyl cyclase was significantly increased in mice, suggesting that cyclic AMP might play an important role in the action of morphine on the central nervous system. Since methadone is employed in the treatment of narcotic addiction and its use has recently been increased by almost 7-fold (Lennard, Epstein & Rosenthal, 1972), we were prompted to investigate the influence of morphine addiction, withdrawal and subsequent methadone replacement on the activities of adenyl cyclase and phosphodiesterase in three different regions of rat brain. Our results show that neither acute nor subacute morphine treatment significantly altered adenyl cyclase and phosphodiesterase activities in cerebral cortex, cerebellum and thalamus-hypothalamus. However, withdrawal of narcotic treatment in animals subacutely treated with morphine markedly decreased the activity of both the basal- and the fluoride-stimulated form of adenyl cyclase in all 3 brain regions. Furthermore, replacement with methadone in animals subacutely treated prevented the observed withdrawal-induced changes in brain cyclic AMP metabolism.

Male Sprague-Dawley rats, 200 g, were used in acute studies whereas subacute experiments were made in animals of approximately 100 g. For examining the effects

Table 1. *Effect of morphine given acutely on adenyl cyclase and phosphodiesterase activities in three regions of rat brain.*

Brain area	Enzyme examined		Treatment: Control	Treatment: Morphine
Cerebral cortex	Adenyl cyclase	−F	35·5 ± 4·9* (100)	27·5 ± 4·7 (77)
		+F (10 mM)	67·5 ± 4·9 (100)	65·5 ± 11·3 (97)
	Phosphodiesterase		75·6 ± 3·0 (100)	77·4 ± 4·8 (102)
Cerebellum	Adenyl cyclase	−F	42·7 ± 3·7 (100)	37·7 ± 6·0 (88)
		+F (10 mM)	50·3 ± 3·1 (100)	52·6 ± 3·9 (105)
	Phosphodiesterase		20·4 ± 2·4 (100)	18·6 ± 1·8 (91)
Thalamus-hypothalamus	Adenyl cyclase	−F	37·0 ± 5·6 (100)	31·3 ± 4·8 (85)
		+F (10 mM)	41·8 ± 6·0 (100)	48·2 ± 6·4 (115)
	Phosphodiesterase		48·6 ± 6·0 (100)	48·0 ± 2·4 (100)

* Means ± S.E. represent 6 animals in each group. Morphine (15 mg kg^{-1}) was administered subcutaneously and rats were killed 1 h later. Adenyl cyclase is expressed as pmol of cyclic AMP formed per mg per min whereas phosphodiesterase activity is given as μmol of cyclic AMP metabolized per mg of tissue per min. Data are also given in percentages (in parentheses) taking the values of control animals as 100%.

F = NaF.

of acute treatment, morphine sulphate (15 mg kg^{-1}) was dissolved in physiological saline, injected subcutaneously in a volume of 0·1 ml and rats killed 1 h later. Addiction to morphine was produced in 18 rats according to the method described by Takemori (1960) as follows: animals were injected twice daily with the 15 mg kg^{-1} dose of morphine i.p. for the 1st week, 30 mg kg^{-1} during the 2nd week and 45 mg kg^{-1} in the 3rd week of treatment. After 21 days of narcotic administration, one group (addicted-control) was killed immediately while the 2nd group (addicted-withdrawn) was maintained without any treatment for an additional 48 h. The 3rd group of addicted rats received methadone (15 mg kg^{-1}) i.p. twice daily for 48 h after the 3-week morphine treatment and constituted the "addicted methadone group." Control animals were given an equal volume of the vehicle. All rats were decapitated and their brains rapidly excised in a cold room at 4°. The cerebral cortex, cerebellum and thalamus-hypothalamus were quickly weighed and 5% homogenates prepared as reported earlier (Hetenyi & Singhal, 1973). Adenyl cyclase and phosphosdiesterase activities were assayed in the homogenates as described previously (Hetenyi & Singhal, 1973; Thomas & Singhal, 1973). Sodium fluoride (10 mM) was added to the incubation medium so that the fluoride-activated form of adenyl cyclase could be measured. All assays were conducted under strictly linear kinetic conditions and adenyl cyclase activity was expressed as pmol cyclic AMP formed per mg tissue per min. In contrast, the activity of brain phosphodiesterase is given as μmol of substrate metabolized per mg tissue per min. The data were subjected to statistical evaluation

Table 2. *Effect of morphine addiction, withdrawal and methadone replacement on brain adenyl cyclase and phosphodiesterase.*

Brain region	Enzyme studied		Treatment: Control	Addicted control	Addicted withdrawn	Addicted methadone
Cerebral cortex	Adenyl cyclase	−F	35·5 ± 4·9** (100)	29·3 ± 5·6 (83)	16·3 ± 4·9 (46)*	25·3 ± 3·0 (71)
		+F (10 mM)	67·2 ± 4·9 (100)	66·6 ± 9·8 (99)	34·3 ± 5·9 (51)*	55·7 ± 2·9 (83)
	Phosphodiesterase		73·5 ± 3·0 (100)	68·4 ± 1·8 (93)	64·8 ± 1·2 (88)	68·2 ± 3·6 (93)
Cerebellum	Adenyl cyclase	−F	42·7 ± 3·7 (100)	39·4 ± 7·7 (92)	27·3 ± 4·5 (64)*	37·0 ± 2·9 (87)
		+F (10 mM)	50·3 ± 3·1 (100)	40·7 ± 8·5 (81)	28·1 ± 6·8 (56)*	36·4 ± 2·4 (72)*
	Phosphodiesterase		18·0 ± 1·2 (100)	19·8 ± 1·8 (111)	18·6 ± 0·6 (103)	24·7 ± 6·0 (137)
Thalamus-hypothalamus	Adenyl cyclase	−F	37·0 ± 5·6 (100)	29·5 ± 4·6 (80)	18·1 ± 3·6 (49)*	24·1 ± 2·0 (65)*
		+F (10 mM)	41·8 ± 6·0 (100)	41·5 ± 4·2 (99)	19·8 ± 1·4 (47)*	37·8 ± 3·4 (90)
	Phosphodiesterase		46·8 ± 1·2 (100)	47·8 ± 2·4 (102)	46·6 ± 1·2 (100)	48·4 ± 1·8 (103)

** Means ± s.e. represent at least 6 animals in each group. For experimental details, see text. Adenyl cyclase is expressed as pmol of cyclic AMP formed per mg per min whereas phosphodiesterase activity is given as μmol of cyclic AMP metabolized per mg tissue per min. Data are also given in percentages (in parentheses) taking the values of control of control rate as 100%.

* Statistically significant difference when compared with the values of control animals injected with saline ($P > 0·05$).

F = NaF.

and significant differences between means calculated as P values. No statistical significance is indicated when the P value was $>0{\cdot}05$.

Data in Table 1 show that 1 h after the injection of morphine (15 mg kg^{-1}), no significant change could be noted in the activity of both basal- and fluoride-stimulated forms of adenyl cyclase in either cerebral cortex, cerebellum or thalamus-hypothalamus. In addition, the activity of the cyclic AMP-degrading enzyme, phosphodiesterase remained unaltered in rats treated acutely with this narcotic. Results in Table 2 show that morphine addiction also failed to produce any significant change in the activity of adenyl cyclase and phosphodiesterase of all 3 brain regions examined. However, brief withdrawal (48 h) from morphine treatment significantly decreased both the basal- and the fluoride-stimulated form of adenyl cyclase in cerebral cortex, cerebellum and thalamus-hypothalamus. Table 2 also shows that in general, methadone replacement in morphine-addicted animals prevented the biochemical changes seen in rats constituting the addicted-withdrawn group except in the case of thalamus-hypothalamic (basal) and cerebellar (fluoride-activated) adenyl cyclase. It is of interest that the activity of brain phosphodiesterase remained completely unchanged in all of the groups investigated.

The present study demonstrates that morphine given either acutely or subacutely, fails to alter the activities of adenyl cyclase and phosphodiesterase in cerebral cortex, cerebellum and thalamus-hypothalamus. In addition, narcotic treatment did not significantly affect the endogeneous levels of cyclic AMP in these three distinct neural regions (unpublished data). Our data also show that withdrawal of morphine treatment from addicted rats significantly decreased the activity of adenyl cyclase in various areas of the brain. Chou, Ho & Loh (1971) had earlier shown that the adenyl cyclase of mouse cerebral cortex was depressed during sub-acute treatment with morphine. It is of interest that Naito & Kuriyama (1973) found that whereas acute morphine did not alter the cerebral adenyl cyclase and phosphodiesterase in mice, chronic narcotic treatment produced a slight rise in the activity of the cyclic AMP-synthesizing enzyme. In addition, Chou & others (1971) demonstrated that morphine given acutely failed to alter cerebellar and hypothalamic adenyl cyclase in mice. However, an elevation in the activity of cerebrocortical adenyl cyclase was observed 1 h after morphine injection. Although it is difficult, at present, to reconcile the data of various workers on the influence of morphine on brain cyclic AMP, the present investigation suggests that methadone replacement in narcotic-addicted animals can prevent the changes observed in neuronal adenyl cyclase activity of rats subjected to withdrawal from morphine.

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Department of Pharmacology,
Faculty of Medicine,
University of Ottawa,
Ottawa, Ontario, Canada,
K1N 6N5

R. L. SINGHAL
S. KACEW
R. LAFRENIERE

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REFERENCES

CHOU, W. S., HO, A. K. S. & LOH, H. H. (1971). *Proc. West. Pharmac. Soc.*, **14**, 42–46.
HETENYI, G. & SINGHAL, R. L. (1973). *Horm. Metab. Res.*, **5**, 139.
LENNARD, H. L., EPSTEIN, L. J. & ROSENTHAL, M. S. (1972). *Science*, **176**, 881–884.
NAITO, K. & KURIYAMA, K. (1973). *Japan. J. Pharmac.*, **23**, 274–276.
TAKEMORI, A. E. (1960). *J. Pharmac. exp. Ther.*, **130**, 370–374.
THOMAS, J. A. & SINGHAL, R. L. (1973). *Biochem. Pharmac.*, **22**, 507–511.