Stereospecific suppression by opiates of the quasi-morphine abstinence syndrome elicited by 3-isobutyl-l-methylxanthine (IBMX)

H.O.J. COLLIER & D.L. FRANCIS*

Research Department, Miles Laboratories Limited, Stoke Poges, Slough

Methylxanthines elicit from otherwise untreated rats several of the behavioural signs seen in the morphine abstinence syndrome (MAS). Heroin readily suppresses the signs elicited by methylxanthines, and naloxone increases their intensity and elicits other signs, such as jumping, to produce a behavioural pattern scarcely distinguishable from the MAS (Collier, Francis, Henderson & Schneider, 1974; Francis, Roy & Collier, 1975). We have, therefore, called the behaviour elicited by xanthines the quasi-morphine abstinence syndrome (QMAS). The present work examines whether suppression by opiates of the QMAS is stereospecific and whether drugs modifying it are effective when injected into a cerebral ventricle.

Drugs were injected into male white Wistar rats (110-200 g) either subcutaneously (s.c.) or, through an indwelling cannula, into the left lateral cerebral ventricle (i.c.v.). The QMAS was elicited with 3-isobutyl-1-methylxanthine (IBMX, 15 mg/kg s.c.) and effects on behaviour were observed 'blind' for up to 15 min after treatment with coded solutions. Fourteen behavioural signs –jumping, teeth chattering, squeak on touch, squeak on handling, diarrhoea, chewing, ptosis, body shakes, head shakes, paw tremor, rearing, restlessness, salivation and licking the penis–were recorded. Total 'quasi-abstinence score' was obtained by counting 1 for presence and 0 for absence of each sign, and was expressed as a median value. The significance of differences between scores was determined by the Mann-Whitney U test.

Heroin (30-300 μg/kg s.c. or 1 and 10 μg/rat i.c.v.) overcame the QMAS in a dose-related way. When the QMAS had been suppressed with heroin (300 μg/kg s.c.), naloxone (10-100 μg/kg s.c. or 0.1-10 μg/rat i.c.v.) reversed the suppression in a dose-related way. Levorphanol (10-100 μg/kg s.c.) also suppressed the QMAS; but dextrorphan was ineffective at 8 mg/kg s.c. These effects were statistically significant at P values ranging downwards from $P = 0.027$ to $P < 0.001$.

That the potent effect of opiates in overcoming the QMAS due to IBMX is stereospecific and is reversed by very small doses of naloxone shows that this is an opiate agonist action. Methylxanthines in turn antagonize opiate agonist actions (Bellville, 1964; Ho, Loh & Way, 1973). This mutual antagonism may provide a clue to the mechanisms of action of both types of drug and to the mechanisms of dependence.

These findings also offer a sensitive *in vivo* method of detecting and estimating the behavioural effects of morphine-like, methylxanthine-like or naloxone-like substances.

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References

BELLVILLE, J.W. (1964). Interaction of caffeine and morphine on respiration. *J. Pharmac. exp. Ther.*, **143**, 165-168.

COLLIER, H.O.J., FRANCIS, D.L., HENDERSON, G. & SCHNEIDER, C. (1974). Quasi-morphine abstinence syndrome. *Nature, Lond.*, **249**, 471-473.

FRANCIS, D.L., ROY, A.C. & COLLIER, H.O.J. (1975). Morphine abstinence and quasi-abstinence effects after phosphodiesterase inhibitors and naloxone. *Life Sci.*, **16**, 1901-1906.

HO, I.K., LOH, H.H. & LEONG WAY, E. (1973). Effects of cyclic 3',5'-adenosine monophosphate on morphine tolerance and physical dependence. *J. Pharmac. exp. Ther.*, **185**, 346-357.

Physiological aspects of the hypnotic properties of steroid hormones

MARGARETHE HOLZBAUER

Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

Although it has been known for nearly half a century that some biogenic steroids possess hypnotic potencies the physiological significance of this effect is still debated. Research in this field was mainly directed towards the development of anaesthetic agents for clinical use and a large number of steroids have been tested for their central depressant activities. They were low in those steroids with high conventional "hormonal activities". However they were very high in some of the hepatic catabolites of steroid hormones which are reduced in ring A and are deprived of

their original "hormonal activities". Some of these compounds, e.g., pregnanolone (a metabolite of progesterone) or the ring A-reduced form of deoxycorticosterone were in some tests up to ten times more active than barbiturates. In the liver, these metabolites are however also esterified, become less lipid soluble and lose hypnotic activity. Interest in the physiology of the hypnotic properties of steroids was revived when it was found that they can be produced and secreted into the blood stream at sites from where they can reach the brain without having to pass first through the liver. For example, the ovary of the rat can produce several ring A-reduced steroids in quantities similar to or even larger than those of progesterone (Holzbauer, 1969, 1971a; Holzbauer & Mason, 1970; Ichikawa, Morioka & Sawada, 1971). Their rate of production and secretion varies during the oestrous cycle and it has been suggested that phase dependent changes in mood or behaviour during the sexual cycle can be caused by these reduced steroids (Holzbauer, 1971b). In addition it may be possible that they depress the activity of those hypothalamic neurons which stimulate the release of the luteinizing hormone releasing factor and may thus play a major role in a feed back system. Observations on their secretion during pregnancy and other factors which influence their production rate will be discussed.

References

HOLZBAUER, M. (1969). Pregnenolone and metabolites of progesterone in the ovary. *J. Physiol.*, **204**, 8-10P.

HOLZBAUER, M. (1971a). Ovarian secretion of steroids with central depressant actions. *J. Physiol.*, **215**, 16P.

HOLZBAUER, M. (1971b). *In vivo* production of steroids with central depressant actions by the ovary of the rat. *Br. J. Pharmac.*, **43**, 560-569.

HOLZBAUER, M. & MASON, P. (1970). Increase in the ovarian content of progesterone metabolites during late proestrus in the rat. *J. Physiol.*, **210**, 128-130P.

ICHIKAWA, S., MORIOKA, H. & SAWADA, T. (1971). Identification of the neutral steroids in the ovarian venous plasma of LH-stimulated rats. *Endocrinology*, **88**, 372-383.

γ-Aminobutyric acid metabolism and the anticonvulsant action of ethanolamine-o-sulphate and di-n-propylacetate

G.M. ANLEZARK, R.W. HORTON*, B.S. MELDRUM, M.C.B. SAWAYA & J.D. STEPHENSON

Departments of Neurology and Pharmacology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

We have evaluated the anticonvulsant effects of ethanolamine-o-sulphate (EOS) and di-n-propyl acetate (DPA) in two test systems, audiogenic seizures in genetically susceptible mice (DBA/2, 21-25 days old) and picrotoxin-induced seizures in chicks (5-6 days old, Rhode Island Red) and correlated changes in behaviour and seizure response to changes in brain γ-aminobutyric acid (GABA) concentration and the activity of enzymes involved in GABA catabolism.

In mice EOS was administered intracerebroventricularly 24 h before test. At 7.5 mg/kg 50% of the animals and at 15 mg/kg 80% showed mild to moderate ataxia and were completely protected against the convulsant effect of auditory stimulation. The remainder showed no behavioural effects, and were not protected or showed partial protection against audiogenic stimulation. GABA transaminase (GABA-t) activity was inhibited 54% and 68% and cerebral GABA concentration increased 4 and 10 fold, after 7.5 and 15 mg/kg respectively. Succinic semialdehyde dehydrogenase (SSADH) activity was not altered.

In chicks, EOS (300 or 600 mg/kg given i.p. 48 h earlier) inhibited GABA-t activity by 54-59% and doubled brain GABA concentration but only raised the ED_{50} for picrotoxin seizures by 30-40% (not significant).

In mice, DPA was administered i.p. 45 min before test. Seizure responses were unaffected at 200 mg/kg, severely reduced at 400 mg/kg and completely absent at 600 mg/kg. Slight behavioural effects were seen only after 600 mg/kg. GABA concentration and GABA-t activity were unchanged after 200 or 400 mg/kg. GABA concentration was increased by 57% and GABA-t activity inhibited by 33% after 600 mg/kg. SSADH activity was unchanged.

In chicks, DPA (400 or 800 mg/kg i.p., 0.5-1 h before test) produced a 33% or 100% increase respectively in the ED_{50} for picrotoxin seizures. Brain GABA concentration was increased 16-30%, but GABA-t was inhibited only by 4-6%.

DPA is claimed to be a competitive inhibitor of GABA-t *in vitro* (Simler, Ciesielski, Maitre, Randrianarisoa & Mandel, 1973). Our results with mouse brain homogenates show that DPA is a poor