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Pharmacological Modulation of Opiate-Like Peptide Systems

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HERZ, A. *Pharmacological modulation of opiate-like peptide systems.* PHARMAC. BIOCHEM. BEHAV. **13**: Suppl. 1, 265–268, 1980.—Our presently somewhat limited knowledge of the modulation of the content, release and turnover of endorphins in brain and pituitary by acute and chronic drug treatment is reviewed and discussed particularly in relation to the problem of addiction. In vitro studies in striatal slices and isolated anterior and intermediate/posterior lobes of the pituitary point to the existence of specific interactions between endorphins and neurotransmitters. In vivo studies have revealed acute GABA-mediated effects of benzodiazepines upon striatal levels of met-enkephalin activity. Morphine exerts no acute effects upon endorphin levels, but decreases the levels of particular endorphins in specific areas of brain and pituitary after long-term treatment; somewhat similar effects are observed after prolonged intake of ethanol, whereas chronic haloperidol treatment results in an increase in levels of endorphins in brain and pituitary. Incorporation studies employing the intermediate/posterior lobe of the pituitary have revealed that the changes in β-endorphin levels produced by prolonged treatment with morphine or haloperidol reflect a respective depressed or enhanced synthesis of the β-endorphin precursor pro-opiocortin, whilst the enzymatic processing of this precursor remains unmodified. Studies in cell-free preparations demonstrated that m-RNA extracted from the intermediate/posterior lobes of chronically morphinized rats possesses a decreased "activity".

Morphine Ethanol Endorphins Enkephalins Endorphin precursor Pituitary

SINCE the detection of endogenous ligands (endorphins) of the opiate receptors in mammalian central nervous system (CNS), pituitary and peripheral organs, much evidence has accumulated in support of a neurotransmitter, neuromodulator and/or neurohormonal function of these peptides. Nevertheless, neither the mediator functions nor the physiological significance of the endorphins are, as yet, well understood. Studies concerning the content and the release of endorphins in particular tissues, and the modulation of endorphin content and release by pharmacological treatment offer one approach towards the resolution of these questions. In the present paper concerning pharmacological modulation of endorphinergic systems, emphasis will be placed upon those changes induced by chronic drug treatment, particularly with opiates and other addictive drugs, in view of the fact that such information may enhance our understanding of the basic processes underlying the phenomenon of addiction.

RELEASE OF ENDORPHINS IN VITRO

Brain

Several groups demonstrated a potassium- or veratridin-evoked Ca^{++}-dependent release of enkephalin from both striatal or globus pallidus slices or from synaptosomal preparations [9, 15, 20, 25]. These findings are supportative of the hypothesis of a neurotransmitter function of enkephalin within the CNS. However, there is, as yet, little information available as to whether in vitro release of enkephalins can be modulated by (other) neurotransmitters or drugs as has been established to occur for certain other neurotransmitters (see [27]). Therefore, we have investigated this question by use of a highly sensitive radioimmunoassay for met-enkephalin, which allows for the monitoring of its release from superfused striatal slices [22]. Using submaximal concentrations of potassium (22 mM), a significant GABA-induced inhibition of met-enkephalin immunoreactivity (M-EI) release was observed; this effect was mimicked by the GABA receptor agonist, muscimol, and inhibited by the GABA antagonist picrotoxin, indicating its mediation by GABA receptors. A series of other putative neurotransmitters or receptor agonists (acetylcholine, carbachol, serotonin, noradrenaline, glutamic acid, substance P) proved ineffective in modulating this potassium-evoked release. This is suggestive that the GABA-induced inhibition is specific in nature and possibly reflects an inhibitory action of striatal GABAergic neurones, probably interneurones, upon enkephalinergic neurones in the striatum.

A relationship of a different kind appears to exist between enkephalinergic neurones and dopaminergic neurones in the striatum. Recently, it has been shown that chronic treatment of rats with the dopamine receptor antagonist haloperidol increases the striatal content of enkephalin [13]. We have been able to confirm this effect of chronic neuroleptic treatment and, in addition, found that the potassium-evoked release of M-EI was increased in slices obtained from such animals, indicative that this higher enkephalin content is due not to a decreased release, but rather to an enhanced synthesis and a consequent increase in the size of the releasable pool. This supposition is consistent with other data pointing to an increased turnover under such conditions [14].

In view of the postulated presence of opioid receptors upon the terminals of enkephalinergic neurones and the possible involvement of such receptors in the regulation of enkephalin release [16], the effect of opiates upon the potassium-evoked release was studied. However, no changes in enkephalin release could be obtained by application of either morphine (10 μM) or naloxone (2 μM) to striatal slices obtained from naive rats. Moreover, no mobilization of enkephalin was seen when naloxone was added to slices from tolerant/dependent rats. These results do not support the hypothesis of the existence of opioid autoreceptors within this tissue (see also below in the discussion of long-term opiate effects).

A potassium-evoked, calcium-dependent release of β-endorphin from rat hypothalamic slices has also been achieved consonant with the view that β-endorphin may act as a neuromodulator/neurotransmitter [21].

Pituitary

The release of endorphins from the anterior lobe of the rat pituitary, which contains approximately equimolar amounts of β-lipotropin and β-endorphin, appears to occur in a different fashion to that observed in the intermediate lobe which contains predominantly β-endorphin. Thus, a calcium-dependent, potassium-evoked release of β-endorphin/β-lipotropin was observed in the anterior lobe, but not in the intermediate/posterior lobe. Further differences were also apparent: addition of either lysine-vasopressin, hypothalamic extracts or noradrenaline increased release from the anterior lobe [24]. (Comparable results were obtained in tissue culture preparations of anterior lobe cells [28].) The intermediate/posterior lobe proved refractory to the above manipulations. Spontaneous outflow from this lobe was, however, in excess of that revealed by the anterior lobe, this release being inhibited by both dopamine and dopamine receptor agonists. Indeed, the presence of a tubero-infundibular dopaminergic pathway running from the hypothalamus to the intermediate lobe has been established, and it is considered that this pathway acts in an inhibitory fashion. The functional significance of this inhibitory dopaminergic input is, as yet, only poorly understood.

IN VIVO DRUG-INDUCED CHANGES IN ENDORPHIN CONTENT AND RELEASE

In general, determination of levels of neurotransmitters/neuromodulators provides only limited information as to the functional status of a mediator system. Thus, a decrease in content may indicate an enhanced release and/or decreased synthesis. Notwithstanding the efforts of a number of laboratories, our present knowledge as to the nature of the turnover of the various endorphins is disappointingly minimal. Nevertheless, certain data have recently become available which may help in the interpretation of the information available concerning the modulation of endorphin content by acute and chronic drug treatment.

Benzodiazepines

In view of a possible relationship between striatal enkephalin and GABAergic neurones as revealed by in vitro studies (see above), the effect of benzodiazepines (possible GABA agonists or potentiators of GABA action) upon endorphin systems was investigated in vivo. A dose-dependent decrease in M-EI of the striatum and an increase in that of the hypothalamus was detected after IV injection of diazepam or other pharmacologically active benzodiazepines in rats [4]. The increase in enkephalin content in the hypothalamus relative to control animals was interpreted as an anti-stress effect in that benzodiazepines prevent a decrease caused by the (stressful) IV injection, which involves immobilization of the rat. The decrease of the M-EI content of the striatum, which was present within a few minutes post-injection, apparently involves GABA-ergic mechanism as this effect is mimicked by both the GABA agonist muscimol and the GABA transaminase inhibitor amino-oxyacetic acid and is blocked by the GABA antagonist bicuculline [5]. The question arises as to whether the diazepam-induced decrease in enkephalin content is due to a release from neuronal terminals or to an increased intraneuronal breakdown of the peptides. The finding that intraventricular administration of the proteinase inhibitor bacitracin prior to injection of diazepam substantially potentiated the antinociceptive effect of diazepam and that this potentiation was partially reversed by naloxone, supports the view that benzodiazepines release enkephalins in the striatum and also possibly in other brain areas [30].

Chronic application of diazepam induced an increase in enkephalin levels only in the striatum and not in other brain areas. In these animals, acute diazepam administration was ineffective in changing striatal content of enkephalin pointing to the occurrence of adaptive changes. On the whole, our understanding of the interaction between the enkephalinergic and GABAergic systems and possible clinical implications of this interaction is rather incomplete.

Opiates

It has been postulated that a prolonged stimulation of opiate receptors might lead to a feedback inhibition of endorphin synthesis [7,16]. Such a phenomenon is known to occur for other neurotransmitter systems. A depressed synthesis may result in a deficiency of endorphins at their receptors contributing, perhaps, to the appearance of withdrawal signs after interruption of the supply of exogenous opiates.

The majority of the studies, which address this possibility, were unable to find significant changes in the enkephalin content of various rat brain areas after either conventional chronic morphine treatment (3–10 days) or naloxone-precipitated withdrawal of the animals [2, 6, 29]. Interestingly, a limited (ca 20%) decrease in enkephalin levels apparent 24 hr after withdrawal was recently reported [1]. Such generally negative results do not, however, exclude the possibility that morphine dependence is associated with an alteration in the turnover of enkephalin in the brain. Indeed, an activation of an enkephalinase has been detected in the brain of chronically morphinized rats [18].

Chronic opiate treatment appears to produce significant changes in β-endorphinergic systems. Although treatment of rats with morphine pellets for a period of 10 days caused no significant changes in the β-endorphin content of the pituitary, hypothalamus or plasma, precipitation of withdrawal by naloxone decreased β-endorphin immunoreactivity (β-EI) of the anterior pituitary lobe and elevated plasma levels of β-EI. These changes should probably be considered as a reflection of the stress of withdrawal.

A different pattern of results was obtained upon prolongation of the period of exposure to morphine from 10 days to one month or more. This long-term treatment resulted in a large (50%) decrease in the β-endorphin content of the intermediate/posterior lobe without affecting that in the anterior

lobe. This decrease in the content of β-endorphin in the intermediate/posterior lobe was parallelled by a decrease in that of α-MSH [23]. In view of the fact of the existence of a common precursor of β-lipotropin/β-endorphin and ACTH/α-MSH [17], these parallel changes are not surprising. Interestingly, a reduction of plasma levels of β-EI was also discovered after long-term administration of morphine [19]. Similarly depressed ACTH-levels have been observed in morphinized animals.

In an attempt to determine the specificity of these changes, an investigation was performed in which opiate agonists exhibiting high receptor affinity, e.g. levorphanol and etorphine, were applied (as pellets) to rats for a period of 4 weeks. Although, these rats developed a high degree of tolerance, no significant changes of β-endorphin in the pituitary or in the brain could be found [31]. These data render questionable the contention that the above changes in endorphin levels, initiated by long-term treatment, are a feature inherent to opiates in general and are caused by a feedback inhibition of endorphin synthesis. This scepticism is in line with the negative results obtained from in vitro studies (see above).

Ethanol

There is, as yet, very little information available concerning changes in endorphinergic systems induced by acute or chronic intake of ethanol. Results, recently obtained in our laboratory [26], revealed some similarities between the changes observed after chronic ethanol intake and after long-term morphine treatment. Whilst acute ethanol administration in rats increased levels of met-enkephalin in the striatum and those of β-endorphin in the hypothalamus, continuation of ethanol intake for 3 weeks revealed in a decrease in the enkephalin content of most brain areas. A particularly high decrease (70%) was observed in the intermediate/posterior lobe of the pituitary, the decrease in the anterior lobe being considerably less pronounced. Comparable changes were also seen in guinea pigs. A complete reversal was observed after 2 weeks.

Neuroleptics

Changes in some way opposite to those observed after long-term administration of morphine and intake of ethanol are induced by chronic neuroleptic treatment. Thus, the considerable increase of enkephalin in the striatum after prolonged chronic haloperidol treatment [13,22] is accompanied by an increase in the β-endorphin content of particular brain areas (hypothalamus, septum) and in the intermediate/posterior lobe of the pituitary. This increase in the intermediate/posterior lobe was parallelled by a considerable increase in ACTH-immunoreactivity (α-MSH) in this area [12]. As with morphine, the β-endorphin content of the anterior lobe remained unchanged.

POSSIBLE MECHANISMS INVOLVED IN THE CHANGES IN ENDORPHIN LEVELS PRODUCED BY CHRONIC DRUG TREATMENT

In an attempt to obtain more information as to the mechanisms underlying the observed changes in endorphin content, incorporation of labelled amino acids into endorphins was studied. The considerable changes observed, particularly in the intermediate/posterior lobe, and the fact that the β-endorphin 31-K precursor, pro-opiocortin, is present in this tissue in a high concentration suggested the use of isolated intermediate/posterior lobes for these studies. A pulse-chase technique, in combination with immunoprecipitation and SDS-gel electrophoresis of labelled products was employed [11].

Intermediate/posterior pituitary lobes obtained from chronically (30 days) morphinized rats displayed a significantly reduced synthesis of the 31-K precursor as compared to placebo-treated controls. Post-translational processing of the pro-opiocortin into β-lipotropin and β-endorphin did not differ significantly. Examination of the intermediate/posterior lobes of rats treated chronically with haloperidol, revealed an enhanced formation of the precursor; the enzymatic processing of the precursor was, similarly, not significantly affected [11,12].

In further experiments, the mechanism of the changes in the formation of pro-opiocortin was analysed. Isolation of m-RNA from the intermediate/posterior lobes of chronically morphinized rats, followed by synthesis of the 31-K precursor in a rabbit-reticulocyte cell-free system, revealed a lower "activity" of the m-RNA from the morphinized animals. Comparison of total protein synthesis with precursor formation showed that the generation of the precursor was depressed to a greater degree than total protein synthesis.

In similar experiments, performed with m-RNA extracted from the intermediate/posterior lobe of rats treated chronically with haloperidol, no significant changes in the activity of m-RNA have been found so far. This may indicate that chronic haloperidol treatment increases the biosynthesis of the 31-K precursor by stimulation of translational processes rather than affecting events at the level of transcription. Experiments involving analysis of the processes underlying the reduced β-endorphin levels observed in the pituitary after chronic ethanol intake are in progress.

In summary, it appears that modulation of the β-endorphin content in the intermediate/posterior lobe by chronic drug treatment is brought about by differing mechanisms. It may be expected that the mechanisms operative in the brain are still more complicated.

IMPLICATIONS FOR THE PROBLEM OF ADDICTION

The expectation, expressed by some, that the detection of endogenous ligands of the opiate receptors might initiate a significant advance in our theoretical understanding and the practical management of the problems of addiction, particularly as concerning opiates, has not, as yet, been fully confirmed. Thus, it has emerged that the dependence liability of endorphins and their synthetic analogues is comparable to those of conventional alkaloid opiates. The fact, however, that chronic intake of addictive drugs may cause a decrease in the activity of endorphinergic systems—although not all data are clearly in agreement with this assertion—is of interest in view of the concept enunciated by Dole and Nyswander [3] that some type of metabolic disease may underly the phenomenon of narcotic addiction. The present data may be interpreted as supportative of such a hypothesis, although the specificity of the changes in endorphin activity induced remains a matter of discussion. In this context, the theory of Goldstein [7] is also worthy of mention, according to which a genetic deficiency in endorphinergic systems might constitute a predisposition to opiate addiction, a theory immensely difficult to convincingly verify or reject. Of interest is the observation that heroin addicts in the process of recovery possess lower levels of plasma β-endorphin as compared to control individuals ([10]; Emrich and Höllt, unpublished ob-

servations). The possibility of the detection and characterization of presently unknown endorphins should be borne in mind. Thus, the recently detected dynorphin 1–13 [8], an opioid peptide exhibiting particularly high activity, behaves quite differently from β-endorphin in several respects and nothing is known, as yet, as to its interactions with addictive drugs or involvement in addictive processes.

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REFERENCES

1. Bergström, L. and L. Terenius. Enkephalin levels decrease in rat striatum during morphine abstinence. *Eur. J. Pharmac.* **60:** 349–352, 1979.
2. Childers, S., R. Simantov and S. H. Snyder. Enkephalin: Radioimmunoassay and radioreceptorassay in morphine dependent rats. *Eur. J. Pharmac.* **46:** 289–293, 1978.
3. Dole, V. P. and M. Nyswander. Heroin-addiction—a metabolic disease. *Archs int. Med.* **120:** 19–24, 1967.
4. Duka, Th., M. Wüster and A. Herz. Rapid changes in enkephalin levels in rat striatum and hypothalmus induced by diazepam. *Naunyn-Schmiedeberg's Arch. Pharmac.* **309:** 1–5, 1979.
5. Duka, Th., M. Wüster and A. Herz. Benzodiazepines modulate striatal enkephalin levels via a GABAergic mechanism. *Life Sci.* **26:** 771–776, 1980.
6. Fratta, W., H. Y. Yang, J. Hong and E. Costa. Stability of met-enkephalin content in brain structures of morphine-dependent or foot shock-stressed rats. *Nature* **268:** 452–453, 1977.
7. Goldstein, A. Opioid peptides (endorphins) in pituitary and brain. *Science* **193:** 1081–1086, 1976.
8. Goldstein, A., S. Tschibana, L. I. Lowney, M. Hunkapiller and L. Hood. Dynorphin (1–13), an extraordinary potent opioid peptide. *Proc. natn. Acad. Sci. U.S.A.* **76:** 6666–6670, 1979.
9. Henderson, G., J. Hughes and H. W. Kosterlitz. In vitro release of leu- and met-enkephalin from corpus striatum. *Nature* **271:** 677–679, 1978.
10. Ho, N. K. K., L. Wen and N. Ling. Beta-endorphin-like immunoreactivity in the plasma of heroin addicts and normal subjects. *Neuropharmacology* **19:** 117–120, 1980.
11. Höllt, V., I. Haarmann, R. Przewłocki and M. Jerlicz. Long-term treatment of rats with morphine decreases in vitro biosynthesis in and release of β-endorphin from intermediate/posterior lobes of pituitary. In: *Neuralpeptides and Neuronal Communication. Advances in Biological Psychopharmacology, Vol. 22,* edited by E. Costa and M. Trabucchi. New York: Raven Press, 1980, pp. 399–405.
12. Höllt, V., R. Przewłocki, M. Bergmann, I. Haarmann and Th. Duka. Increased biosynthesis of β-endorphin in the pars intermedia of rat pituitaries after long-term treatment with haloperidol. In: *Endogenous and Exogenous Opiate Agonists and Antagonists,* edited by E. L. Way. New York: Pergamon Press, 1980, pp. 325–328.
13. Hong, J. S., H.-Y. T. Yang, M. Fratta and E. Costa. Rat striatal methionine-enkephalin content after chronic treatment with cataleptogenic and non cataleptogenic antischizophrenic drugs. *J. Pharmac. exp. Ther.* **205:** 141–147, 1978.
14. Hong, J. S., H.-Y. T. Yan,g, J. C. Gillin, A. M. Di Guilio, W. Fratta and E. Costa. Chronic treatment with haloperidol accelerates the biosynthesis of enkephalin in rat striatum. *Brain Res.* **160:** 192–195, 1979.
15. Iversen, L. L., S. D. Iversen, F. E. Bloom, T. Vargo and R. Guillemin. Release of enkephalin from rat globus pallidus in vitro. *Nature* **271:** 679–681, 1978.
16. Kosterlitz, H. W. and J. Hughes. Some thoughts on the significance of enkephalin, the endogenous ligand. *Life Sci.* **17:** 91–96, 1975.
17. Mains, R. E., B. A. Eipper and N. Ling. Common precursor to corticotropins and endorphins. *Proc. natn. Acad. Sci. U.S.A.* **74:** 3014–3018, 1972.
18. Malfroy, B., J. P. Swerts, A. Guyon, B. P. Roques and J. C. Schwartz. High-affinity enkephalin degrading peptidase in brain is increased after morphine. *Nature* **276:** 522–526, 1978.
19. Millan, M. J., R. Przewłocki, M. Jerlicz, C. Gramsch, V. Höllt and A. Herz. Stress-induced release of brain and pituitary β-endorphin: major role in generation of hyperthermia, not analgesia. *Brain Res.,* in press, 1980.
20. Osborne, H., V. Höllt and A. Herz. Potassium-induced release of enkephalins from rat striatal slices. *Eur. J. Pharmac.* **48:** 219–221, 1978.
21. Osborne, H., R. Przewłocki, V. Höllt and A. Herz. Release of β-endorphin from rat hypothalamus in vitro. *Eur. J. Pharmac.* **55:** 425–428, 1979.
22. Osborne, H. and A. Herz. K^+-evoked release of met-enkephalin from rat striatum in vitro: Effect of putative neurotransmitters and morphine. *Naunyn-Schmiedeberg's Arch. Pharmac.* **310:** 203–209, 1980.
23. Przewłocki, R., V. Höllt, Th. Duka, G. Kleber, Ch. Gramsch, I. Haarmann and A. Herz. Long-term treatment decreases endorphin levels in rat brain and pituitary. *Brain Res.* **174:** 357–361, 1979.
24. Przewłocki, R., V. Höllt, K. H. Voigt and A. Herz. Modulation of in vitro release of β-endorphin from separate lobes of rat pituitary. *Life Sci.* **24:** 1601–1609, 1979.
25. Richter, J. A., D. L. Wesche and R. C. A. Frederickson. K^+-stimulated release of leu- and met-enkephalin: lack of effect of morphine and naloxone. *Eur. J. Pharmac.* **56:** 105–113, 1979.
26. Schulz, R., M. Wüster, Th. Duka and A. Herz. Acute and chronic ethanol treatment changes endorphin levels in brain and pituitary. *Psychopharmacology* **68:** 221–227, 1980.
27. Starke, K. Presynaptic regulation of release in the central nervous system. In: *The Release of Catecholamines from Adrenergic Neurones,* edited by D. M. Paton. Oxford: Pergamon Press, 1979, pp. 143–193.
28. Vale, W., C. Richter, L. Yang, S. Minick and R. Guillemin. Effects of purified hypothalamic corticotropin releasing factor and other substances on the secretion of adrenocorticotropin and β-endorphin immunoreactivities in vitro. *Endocrinology* **103:** 1911–1915, 1978.
29. Wesche, D. L., V. Höllt and A. Herz. Radioimmunoassay of enkephalins. *Naunyn-Schmiedeberg's Arch. Pharmac.* **301:** 79–82, 1977.
30. Wüster, M., Th. Duka and A. Herz. Diazepam-induced release of opioid activity in the rat brain. *Neurosci. Lett.* **16:** 335–337, 1980.
31. Wüster, M., R. Schulz and A. Herz. Inquiry into endorphinergic feedback mechanisms during the development of opiate tolerance/dependence. *Brain Res.* **189:** 403–411, 1980.