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ANTAGONISM OF MORPHINE-INDUCED CATALEPSY BY L-PROLYL-L-LEUCYL-GLYCINAMIDE

SIMON CHIU and RAM K. MISHRA

Neuropharmacology Lab. Departments of Psychiatry and Neurosciences, McMaster University, Hamilton, Ontario L8S 4J9, Canada

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In view of the recently demonstrated extra-endocrine central actions of hypothalamic releasing hormones, we have investigated the effects of prolyl-leucyl-glycinamide (PLG) and thyrotropin releasing hormone (TRH) on morphine-induced catalepsy. Although acute administration of PLG (10 mg kg^{-1} s.c.) slightly attenuated the cataleptic response, chronic PLG treatment (10 mg kg^{-1} s.c. for 10 days) virtually abolished morphine-induced catalepsy. TRH, administered subcutaneously, exhibited little or no anti-cataleptic activity. These results are discussed in relation to the possible central site of narcotic-induced catalepsy and the therapeutic potential of PLG in Parkinson's disease.

L-Prolyl-leucyl-glycinamide (PLG) Thyrotropin releasing hormone (TRH) Morphine Catalepsy Parkinsonism

1. Introduction

Until recently, it was thought that the hypothalamic releasing and inhibiting hormones only subserved the physiological function of modulating the secretion of pituitary hormones; recent increasing evidence suggests that they also exert direct profound actions on the central nervous system in mammals independent of their established hypophysiotropic function (Plotnikoff & Kastin, 1976). The hypothalamic factor inhibiting the release of melanocyte stimulating hormone from the anterior pituitary * (MIF) has been isolated and identified as the tripeptide, L-prolyl-L-leucyl-glycinamide (PLG) (Nair et al., 1971). Since then, it has been shown to potentiate the behavioural effects of L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (Huidobro-Toro et al., 1974) and antagonize the tremor induced by the cholinergic agent oxotremorine, in both the hypophysectomized and sham-operated animals (Plotnikoff et al., 1972). Despite the results of clinical studies indicating that PLG was relatively effective in alleviating at least temporarily, the dyskinesia symptoms of Parkinson's disease which are thought to originate from the degeneration of the nigro-striatal dopaminergic neurons of the basal ganglia (Ehrensing et al., 1977; Barbeau et al., 1976), the ultimate therapeutic potential of PLG as an anti-dyskinesia agent remains yet to be established.

In rodents, drug-induced catalepsy describes an abnormal motor status characterized by the maintenance of atypical body postures as imposed by the experimenter (Munkvad et al., 1968). Since antipsychotics of the phenothiazine and butyrophenone groups are known to produce extrapyramidal Parkinsonian-like

* Throughout the text, Melanocyte Stimulating Hormone release inhibiting Factor (MIF) is referred to as prolyl-leucyl-glycinamide (PLG).

motor deficits in humans (Byck, 1975), neuroleptic (antipsychotic)-induced catalepsy in laboratory animals has been used as the behavioural index to identify potential anti-Parkinsonian agents (Morpungo, 1962). Catalepsy, however, is not confined to the category of neuroleptic drugs. Narcotic analgesics like morphine and methadone have also been demonstrated to produce a cataleptic reaction in rats (Ahtee, 1976). Moreover, it has been recently found that β-endorphin, the endogenous opiate-like peptide isolated from the pituitary, when administered intraventricularly, elicited dose-dependent cataleptic and muscular hypertonic phenomena (Bloom et al., 1976; Jacquet and Marks, 1976; Motomatsu et al., 1977). The observed reversal of β-endorphin-induced catalepsy by the precursor of dopamine, L-DOPA, currently employed in Parkinson's disease therapy, perhaps illustrates the relevance of this behavioural model to Parkinsonism and related neurological deficits. In this respect it is interesting to note that opiate receptors have recently been demonstrated to exist in high concentrations in the basal ganglia involved in the regulation extrapyramidal motor function (Pert et al., 1976). Hence we consider it relevant to evaluate the neuropharmacological profile of activity of PLG in this animal behavioural paradigm of catalepsy. The purpose of the present study was to examine the effects of acute and chronic administration of PLG on morphine-induced catalepsy. In order to delineate the pharmacological specificity of the central actions of PLG we have also included the results obtained from another neuropeptide, thyrotropin releasing hormone (TRH), which has been found to antagonize pentobarbital- and ethanol-induced narcosis (Breese et al., 1974).

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Canadian Breeding Farm, Quebec, Canada) weighing between 200—210 g upon arrival were housed individually in plastic cages and maintained on a 12—12 h light—darkness cycle. They were provided with food and water ad libitum and allowed to acclimatize for at least two days prior to the start of the experiments. All animals were used only once for the study.

2.2. Quantification of cataleptic responses

At each experimental session, the animals were permitted to acclimatize themselves to a sound-attenuated room for at least half-an-hour before the administration of various drugs. The intensity of the cataleptic response was evaluated at specified time intervals after drug treatments and entailed placing both front paws of the animals in extended positions on a horizontal metal bar mounted 10 cm above a wooden platform (Ezrin-Waters et al., 1976). Catalepsy score was measured by the time spent by the animals in maintaining this imposed posture and reported to the nearest second, the maximum score being 120 sec. No attempt was made to evaluate the catatonic effect of morphine as manifested in the degree of concomitant muscular rigidity.

2.3. Drugs

PLG (Sigma Co., U.S.A.), TRH (Beckman, U.S.A.) and morphine sulfate (Ingram & Bell, Canada) were administered by the s.c. route. Saline-control animals received equivalent volumes of isotonic saline. All drug treatments were rendered between 9 am and 6 pm.

2.4. Statistical analysis

Data were analyzed by the non-parametric Mann—Whitney U-test.

3. Results

3.1. MIF and morphine interactions

Fig. 1 shows the time course of the effect of PLG (1—40 mg kg^{-1}) on the intensity of

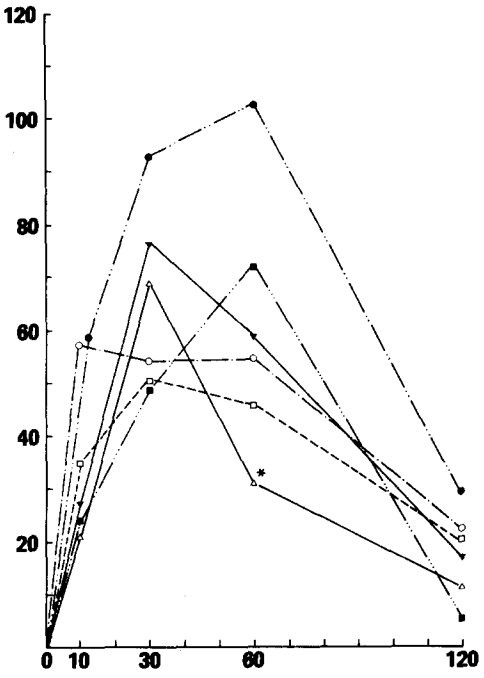

Fig. 1. Dose—response relationship of the effect of pretreatment with PLG (L-Prolyl-leucyl-glycinamide) on morphine-induced catalepsy. Naive rats were injected s.c. with PLG at the dosage of (□) 1 mg kg^{-1} n = 6; (■) 5 mg kg^{-1}, n = 6; (△) 10 mg kg^{-1}, n = 8; (○) 20 mg kg^{-1}, n = 6; (▼) 40 mg kg^{-1}, n = 9, exactly 20 min before s.c. administration of morphine sulfate at the dosage of 30 mg kg^{-1}. Morphine-control animals (●, n = 6) were injected with morphine sulfate (30 mg kg^{-1} s.c.) only. The intensity of the cataleptic response was evaluated at 10 min, 30 min, 60 min and 120 min after morphine treatment as described in the section Materials and Methods. * Significantly different from the corresponding morphine-control, $P < 0.01$. *Ordinate:* catalepsy score (sec); *abscissa:* time (min) after morphine treatment.

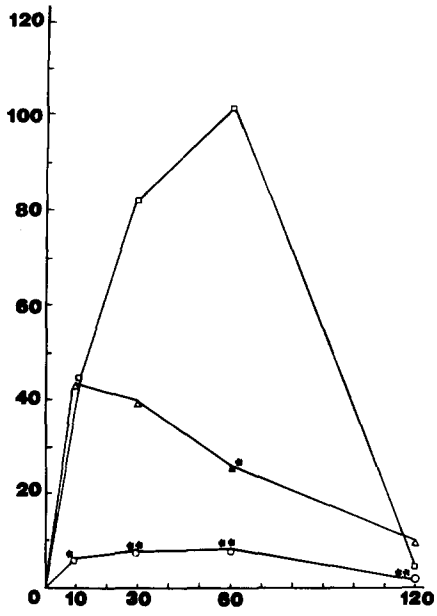

Fig. 2. Effect of chronic PLG treatment on acute morphine-induced catalepsy. 3 groups of rats were subjected to following dosage regimen: (1) ○ group (n = 6) received PLG at the dosage of 10 mg kg^{-1} s.c. for 10 consecutive days and morphine sulfate (30 mg kg^{-1} s.c.) was injected 20 min after the last PLG injection. (2) △ group (n = 6) received PLG in the same manner as ○ group except morphine (30 mg kg^{-1} s.c.) was administered 8 h after the tenth PLG injection. (3) □ group (n = 6) received equivalent volumes of physiological saline solution by the s.c. route and were challenged with morphine sulfate (30 mg kg^{-1} s.c.) 20 min after the last saline injection. Catalepsy testing and statistical analysis of results were conducted as previously described in the section Materials and methods. * Significantly different from the morphine-control at the respective time intervals, $P < 0.05$. ** Significantly different from the morphine-control $P < 0.01$. *Ordinate:* catalepsy score (sec); *abscissa:* time (min) after morphine treatment.

catalepsy caused by acute s.c. morphine administration (30 mg kg^{-1}) in rats. Whereas 1 and 5 mg kg^{-1} were ineffective in preventing the development of catalepsy, 10 mg kg^{-1} significantly reduced the maximum cataleptic response occurring sixty minutes after morphine treatment. Higher dosages of PLG (20 and 40 mg kg^{-1}) failed to decrease the cataleptic action of morphine. The optimal dosage of PLG for reversing morphine-induced catalepsy appeared to fall within the 10—20 mg kg^{-1} range. Earlier studies also indicated that higher doses of PLG were ineffective in the L-DOPA potentiation test, as well as in the reversal of oxotremorine-induced tremor in mice and deserpidine-induced sedation in monkeys (Plotnikoff et al., 1972; 1975). Conceivably, the biphasic anomalous dose—response relationship of PLG may explain the rather narrow dosage range found to be efficacious in ameliorating dyskinesia in humans (Barbeau et al., 1976).

Parenthetically, the concomitant analgesic

effect of morphine as evaluated by the absence of vocalization response to the stimulus of tail pinching with forceps, was virtually unaffected by PLG at all dosages throughout the entire period of observation (data not shown). The selective antagonistic action of PLG against morphine-induced catalepsy, but not against morphine-induced analgesia, appears to support the hypothesis that the opiate-sensitive neurons mediating the antinociceptive and cataleptogenic actions of morphine may, after all, be functionally and spatially distinct (Kaakkola and Ahtee, 1977).

As depicted in fig. 2, chronic administration of PLG at the dosage of 10 mg kg^{-1} for ten consecutive days virtually abolished the cataleptic effects elicited by acute morphine administration (30 mg kg^{-1}). This pronounced central action of PLG evidenced after prolonged treatment contrasted directly with the acute effects of identical dosage of PLG in reversing morphine-induced catalepsy (fig. 1). On the other hand, the cumulative anticataleptic activity of PLG was less marked when the last PLG injection was conducted 8 h prior to acute morphine challenge, indicating that the changes in the sensitivity of central opiate-sensitive cataleptogenic sites induced by this tripeptide are likely to be reversible.

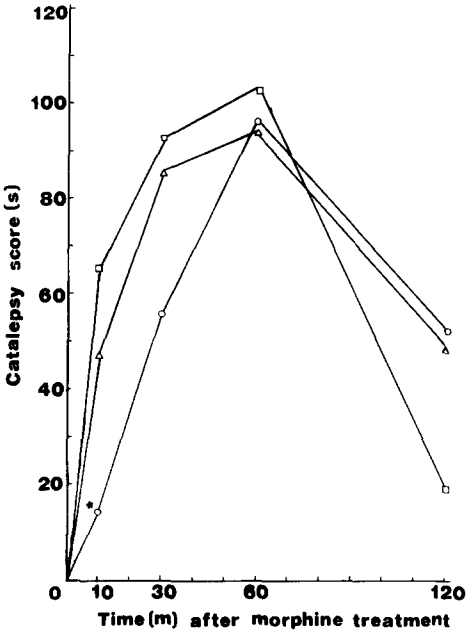

Fig. 3. Effect of TRH pretreatment on morphine-induced catalepsy. TRH was administered i.p. at the dosage (○) 40 mg kg^{-1}, n = 5; (△) 10 mg kg^{-1}, n = 5; exactly twenty minutes prior to morphine administration (30 mg kg^{-1} s.c.). Morphine-control animals (□ n = 6) were administered morphine morphine sulfate (30 mg kg^{-1} s.c.) only. Catalepsy testing and statistical analysis of data were carried out as described in the section Materials and methods. * Significantly different from the corresponding morphine-control, $P < 0.05$. *Ordinate:* catalepsy score (sec); *abscissa:* time (min) after morphine treatment.

3.2. TRH and morphine interactions

As shown in fig. 3, TRH, in contrast to PLG, at the intraperitoneal dosage of 4 mg kg^{-1} produced only a transient, though statistically significant, decrease in the cataleptic response at ten minutes after acute morphine administration.

4. Discussion

In view of the potentiating effect of TRH on oxotremorine-induced tremor in mice detailed in a previous study (Kruse, 1976), it is not surprising to obtain negative results with TRH; furthermore, episodes of "wet dog shakes" were observed to accompany the reversal of β-endorphin-induced depression of motor activity by intraventricular TRH administration. (Tache et al., 1975). More significantly, the demonstrated anticataleptic activity of PLG upon chronic, and, to a lesser extent, acute administration confirms and extends the data from an earlier report on the potent antagonism of fluphenazine-induced catalepsy by PLG (Voith et al., 1977). In both investigations, prolonged treatment of PLG is required to counteract the cataleptic propensities elicited by the prototypal narcotic and neuroleptic. The dual action of PLG in reversing both narcotic- and neuroleptic-induced catalepsy may not be construed as evidence that

the two categories of catalepsy are mediated by the same neuroanatomical substrate. While neuroleptic-induced catalepsy is considered to be a behavioural consequence of decreased dopaminergic neurotransmission in the striatum (Costall and Naylor, 1974a), bilateral lesion of the striatum made either by stereotaxic injection of the specific neurotoxin 6-hydroxydopamine (Nakamura et al., 1973), or by the electrolytic techniques (Koffer et al., 1978), potentiated morphine catalepsy. Over the past few years, however, evidence has accumulated pointing to the functional significance of another dopaminergic neuronal system, the mesolimbic dopaminergic (Kelly and Moore, 1976) system with respect to the control of drug-induced locomotor behaviour (Pycock et al., 1978). In fact, lesion and intracranial injection studies strongly suggest that changes in the neuronal activity of the mesolimbic dopaminergic afferents and terminals may be responsible for morphine-induced akinesia. Micro-injection of morphine into the nucleus accumbens, but not into the nucleus caudatus, elicited catalepsy in rats (Dill and Costa, 1977). Lesion of the nucleus amygdaloidus completely abolished morphine-induced catalepsy, and unmasked sterotyped patterns of behaviour (Costall and Naylor, 1974b). These considerations suggest the PLG interacts with the cataleptogenic sites in both the nigrostriatal and meolimbic dopaminergic neuronal systems in reversing neuroleptic- and narcotic-induced catalepsy. In this respect it is relevant to note that Parkinsonian patients upon autopsy demonstrated loss of dopamine content in the nucleus accumbens comparable to that observed in the nucleus caudatus (Farley et al., 1977).

Attempts to claim dopaminergic agonist properties for PLG have yielded inconsistent results. Although PLG potentiated the mounting behaviour induced by apomorphine (Plotnikoff et al., 1974), no amphetamine-like stereotyped behaviour could be observed in rats (Cox et al., 1976). The enhanced striatal dopamine level and synthesis (Friedman et al., 1973), and the increased dopamine turnover in the nucleus caudatus (Versteeg et al., 1978), are not compatible with the negative results obtained by other investigators (Kostrzewa et al., 1975; Plotnikoff et al., 1975). The demonstrated stringent structural requirement for the amide moiety of PLG for the expression of its biological activity (Johnson and Snissnan, 1978), coupled with the selective autoradiographic localization in neurons (Pelletier et al., 1975), argue cogently for the existence of a distinct receptor for this tripeptide in the mammalian central nervous system.

Alternatively, the antagonistic action of chronic PLG administration on acute morphine-induced catalepsy may be interpreted in the light of its facilitatory effect on certain elements of the memory process considered by some investigators to be operative in the development of tolerance to morphine (Clouet and Iwatsubo, 1975). Previous studies have shown that physiological dosages of PLG facilitated physical dependence on morphine (Van Ree and De Wied, 1976) The extent to which the applicability of the learning paradigm to the cataleptic behaviour response is uncertain, insofar as a high pharmacological dosage of PLG was used in our study, but it is conceivable that interaction of PLG with the nigro-striatal dopaminergic system (Versteeg et al., 1978) contributes either directly or indirectly towards the induction of tolerance to morphine. Furthermore, although the functional significance of the long striatopallidal enkephalinergic pathway, as identified by immunohistochemical techniques (Cuello and Paxinos, 1978), in modulating extrapyramidal motor function remains to be determined, the possibility cannot be excluded that enkephalinergic neuronal activity may participate in the anti-cataleptic action of this tripeptide hormone. The apparent observed lack of affinity of PLG for opiate receptor binding in vitro (Terenius et al., 1975), however, is not necessarily contradictory to the "enkephalin hypothesis" for PLG action, in view of the heterogeneity of central opiate

receptors exhibiting differential affinities for a variety of binding ligands (Lord et al., 1977).

To our knowledge, this is the first study of the anticataleptic activity of PLG against morphine-induced akinesia. The relevance of this experimental finding to the clinical efficacy of PLG as a potential anti-Parkinsonian agent requires further investigation.

Acknowledgements

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