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Haloperidol treatment increases the biosynthesis and release of endorphins in guinea-pig ileum

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Guinea-pigs treated acutely (5 mg kg^{-1} i.p.) and chronically (2 or 5 mg kg^{-1} day^{-1} for 4 days) with haloperidol, show an increase of the inhibitory response produced by electrical stimulation at 10 Hz of the ileum myenteric plexus-longitudinal muscle (MPLM) preparation. The response can be reversed by the specific narcotic antagonist naloxone (5×10^{-7} M), which suggests that the increase in the response produced by haloperidol is mediated by release of endorphins. When haloperidol-treated guinea-pigs received cycloheximide (24 and 12 h before death), there was a substantial decrease in the response indicating that the biosynthesis and release of endorphins are increased by haloperidol.

The guinea-pig ileum myenteric plexus-longitudinal muscle (MPLM) preparation is known to be sensitive to opiates (Creese & Snyder 1975), to bind stereospecifically labelled narcotic agonists and antagonists (Pert & Snyder 1973) and to contain enkephalins (Smith et al 1976). We have used this preparation as a source of endorphins which can be released by electrical stimulation at high frequency (Puig et al 1977, 1978).

Neuroleptics can affect the release and/or biosynthesis of endorphins in the brain of rats (Höllt 1981). Chronic treatment of rats with haloperidol and other neuroleptic drugs over 1–3 weeks significantly increases concentrations of methionine-enkephalin in the corpus striatum and nucleus accumbens (Hong et al 1978). The increased striatal levels are due to an increased biosynthesis of the peptide (Hong et al 1979). Moreover, an increase of pituitary, brain and plasma concentrations of immunoreactive β-endorphin in haloperidol-treated rats has been reported (Höllt & Bergmann 1982).

To obtain an indication of the regulation of the release of endorphins in guinea-pig ileum by other neuronal systems, we have examined the effects of acute and chronic administration of haloperidol on their release in the myenteric plexus. To investigate whether the increased release of endorphins that we found to be caused by haloperidol is due to an increase in the synthesis of these peptides, we have administered cycloheximide (which presumably inhibits polypeptide synthesis by blocking translation) to animals treated chronically with haloperidol or saline and we have measured the release of endorphins in the preparation.

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MATERIALS AND METHODS

Myenteric plexus-longitudinal muscle preparation

Experiments were on the guinea-pig ileum myenteric plexus-longitudinal muscle (MPLM) preparation. Male or female tricolour guinea-pigs (400–550 g) were stunned and decapitated. The abdomen was opened by a midline incision, the terminal ileum removed and the last 10–20 cm discarded. The MPLM preparation was prepared by a combination of methods of Paton & Zar (1968) and Kosterlitz et al (1970). The preparation was suspended in a 2 ml organ bath containing Krebs solution (mM: NaCl, 118; KCl, 4·7; $CaCl_2$, 2·5; $NaHCO_3$, 25; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; glucose, 11) at 37 °C. The bath fluid was bubbled with 95% O_2 and 5% CO_2. The preparation was suspended under a resting tension of 0·3 g and was allowed to equilibrate for 45 min during which time the preparation was washed every 15 min with Krebs solution at 37 °C. The isometric contractions of the muscle were registered by means of a Statham force transducer coupled to a Dinograph Beckman polygraph recorder.

The preparation was stimulated by a Grass stimulator through two platinum ring electrodes placed at the top and bottom with rectangular pulses of 1 ms duration, supramaximal voltage (40 V) and a frequency of 0·2 Hz.

After a 45 min equilibration period, the preparation was stimulated at a frequency of 0·2 Hz (basal frequency of stimulation) for 15 min. To produce an opiate-like inhibition of the contraction, we increased the frequency of stimulation to 10 Hz for 30 s. When the basal frequency of stimulation was resumed, an inhibitory response appeared which was reversed by naloxone. The main component of this response is due to endorphins release (Puig et al

1978). Periods of stimulation at 10 Hz for 30 s were repeated every 30 min, 6 times. The preparation was washed with Krebs solution at 37 °C after 10 min each stimulation. In the 6th stimulation period, naloxone 5×10^{-7} M was added to the bath in a volume of 0·1 ml. The inhibitory response and the reversal produced by naloxone were calculated by the procedure described by Puig et al (1977).

Acute haloperidol treatment

Haloperidol (5 mg kg^{-1}) or 0·9% NaCl (saline) were administered intraperitoneally to different groups of guinea-pigs at a volume of 0·5 ml, and animals were decapited 2 h later.

Chronic haloperidol treatment

In chronic experiments, guinea-pigs were treated for 4 days with daily injections of haloperidol (2 or 5 mg kg^{-1}) or saline intraperitoneally.

Cycloheximide treatment

Guinea-pigs were injected with either saline or haloperidol (5 mg kg^{-1} day^{-1}) intraperitoneally for 4 days and were then given cycloheximide (10 mg kg^{-1} i.p. in saline) 24 and 12 h before death.

Met-enkephalin dose-response curves

To study the possibility that haloperidol or cycloheximide could disturb the opiate receptor of the MPLM preparation, we obtained dose-response curves with met-enkephalin in haloperidol-treated (5 mg kg^{-1} day^{-1} for 4 days), cycloheximide-treated (10 mg kg^{-1} i.p. 24 and 12 h before death) and saline-treated (for 4 days) guinea-pigs. We also determined the reversal by naloxone (5×10^{-7} M) of the inhibitory response elicited by met-enkephalin.

Drugs used were: Naloxone hydrochloride (Endo Laboratories Inc., New York, NY); haloperidol (I.F. Latino, Madrid, Spain); cycloheximide (Sigma Chemical Company, St Louis, MO); methionine-enkephalin (Sigma Chemical Company, St Louis, MO).

Statistical analysis of results was performed by two-way analysis of variance. The criterion for significance was a P value less than 0·05.

RESULTS

Acute haloperidol treatment

The inhibitory response obtained in the haloperidol-treated group was significantly greater than in the saline-treated group ($P < 0·001$). The response in the haloperidol-treated guinea-pigs was 59·2 ± 1·5%, while in the controls it was 42·8 ± 1% (Fig. 1).

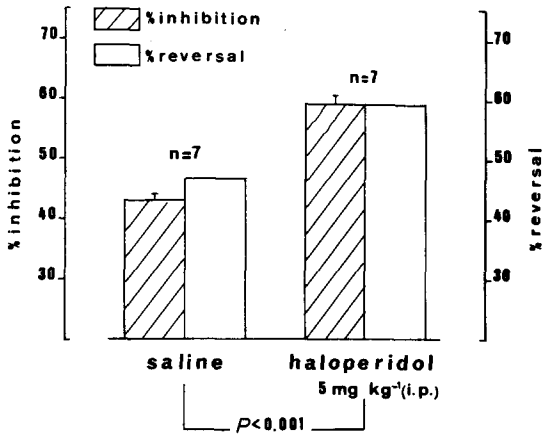

FIG. 1. Per cent inhibitory response (hatched columns) and percentage reversal by naloxone 5×10^{-7} M (open columns) of the MPLM preparation elicited by stimulation at 10 Hz when guinea-pigs were injected with either saline or haloperidol (5 mg kg^{-1} i.p.) 2 hours before death (mean ± s.e.m.).

In the presence of naloxone the response was reversed in both groups, being 59·1 ± 4·5% in the haloperidol-treated group and 46·5 ± 4·1% in the control. In isolated experiments in which guinea-pigs were given a 2 mg kg^{-1} dose of haloperidol 2 h before death, no significant differences in the inhibitory response obtained between drug and saline were noted.

Chronic haloperidol treatment

Treatment with daily injections of haloperidol (2 mg kg^{-1}) for 4 days revealed a significantly higher inhibitory response than that obtained in animals treated with saline for 4 days ($P < 0·001$; Fig. 2). The response obtained was 47·5 ± 1% in the saline-treated group and 57·9 ± 1·7% in the haloperidol-treated group. When the stimulation was performed in the presence of naloxone, the response was reversed in both groups, but the reversal was more pronounced in the haloperidol-treated group. The reversal of the response was 61·2 ± 6·4% in the haloperidol-treated group and 59·9 ± 3·5% in the saline-treated group. When guinea-pigs were treated with a larger daily dose of haloperidol (5 mg kg^{-1}) for 4 days, the inhibitory response obtained was significantly higher than that the saline-treated group for 4 days ($P < 0·001$) and than that of the group treated with haloperidol 2 mg kg^{-1} day^{-1} for 4 days (Fig. 2, Table 1). The response was 66·7 ± 0·6%. The greatest response obtained in this last group was reversed by naloxone to a higher extent (67·8 ± 7·8%) compared with previous groups.

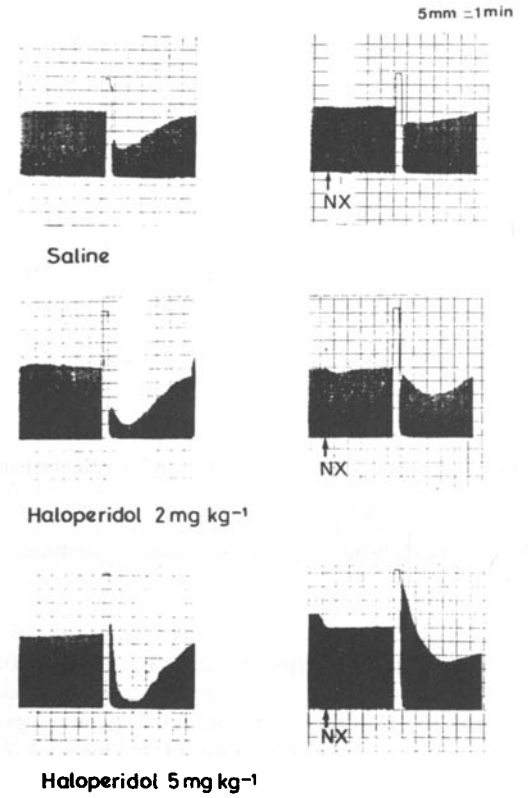

FIG. 2. Examples of the inhibitory response of the MPLM preparation elicited by stimulation at 10 Hz (left) and reversal by naloxone 5×10^{-7} M (right) when guinea-pigs were injected for 4 days before death with saline, haloperidol (2 mg kg^{-1} day^{-1}) or haloperidol (5 mg kg^{-1} day^{-1}) intraperitoneally.

Cycloheximide treatment

Table 1 shows the inhibitory response obtained by stimulation of the preparation and reversal by naloxone when guinea-pigs treated with either saline or haloperidol (5 mg kg^{-1} day^{-1}) for 4 days received injections of cycloheximide (10 mg kg^{-1}) 24 and 12 h before death. In the saline-treated group, the response was unchanged, whereas in the haloperidol-treated group there was a substantial decrease of the response ($P < 0{\cdot}001$) after cycloheximide treatment (Table 1). When the stimulation was performed in the presence of naloxone, there was a decrease of the response in both groups (Table 1).

Met-enkephalin dose-response curves

Dose-response curves to met-enkephalin were obtained in different groups of guinea-pigs previously treated with saline (one injection/day for 4 days), haloperidol (5 mg kg^{-1} day^{-1} for 4 days) and

Table 1. Inhibitory response and reversal by naloxone (5×10^{-7} M) obtained by stimulation at 10 Hz of the MPLM preparation after administration of cycloheximide (10 mg kg^{-1} i.p.) 24 and 12 h before death in haloperidol-** and saline-treated guinea-pigs. Inhibitory response and reversal by naloxone are expressed as percentages. Mean ± s.e.m.; n = 7–14.

	Saline		Haloperidol	
Treatment	% Inhibition	% Reversal	% Inhibition	% Reversal
Vehicle	47·7 ± 1	60 ± 3·5	66·7 ± 0·6	67·8 ± 7
Cycloheximide	50 ± 2	65·8 ± 4	45·6 ± 1*	67·5 ± 5

* $P < 0{\cdot}001$ vs haloperidol-treated without cycloheximide.
** Guinea-pigs received daily injections of haloperidol (5 mg kg^{-1} i.p.) or saline for 4 days.

cycloheximide (10 mg kg^{-1} 24 and 12 h before death). There was no significant difference between the response obtained among the groups. The reversal by naloxone (5×10^{-7} M) of the response elicited by met-enkephalin (5×10^{-7} M) was 87–90% in all three cases.

DISCUSSION

The present investigation has demonstrated that both acute and chronic haloperidol treatments produce in the guinea-pig MPLM preparation a significant increase of the inhibitory response obtained by electrical stimulation at 10 Hz. Previously, Puig et al (1978) have shown that the response obtained by electrical stimulation, at high frequency, of the MPLM preparation, is due mainly to the release of endorphins. Our results would indicate that the increase of the response observed in haloperidol-treated guinea-pigs would be due to an increase in the release of endorphins, produced by the drug. In spite of the high inhibitory response obtained, both in acute and chronic treatment with haloperidol, a great proportion of this response was reversed by naloxone (a specific opiate antagonist). This shows that the increase produced in the response is due to an increase in the release of endorphins at that level, but the exact nature of the released material is unknown.

In acute haloperidol treatment, a dose of 5 mg kg^{-1} was necessary to produce a significant increase in the inhibitory response, as with smaller doses, in isolated tests, no significant variation in the response was observed. When chronic treatment was administered, 2 mg kg^{-1} day^{-1} was sufficient to produce a significant increase of the inhibitory response. The effect of haloperidol was dose-dependent, since the greatest increases in the response were produced with the dose of 5 mg kg^{-1} day^{-1} for 4 days. Moreover, the highest percentages

of reversal by naloxone occurred after haloperidol treatment with this same dosage, a fact that would imply that the greatest release of endorphins is produced when guinea-pigs are treated under these conditions.

From these results we can affirm that the quantity of endorphins released is going to be dependent on the dosage and on the time of administration of haloperidol. Our results agree with studies in the central nervous systems with haloperidol and other neuroleptics, suggesting that endorphins could participate in effects of antipsychotic drugs. An increase in the rat corpus striatum and nucleus accumbens met-enkephalin content was noted after chronic treatment with haloperidol and other dopaminergic blocking (Hong et al 1978). Recent studies have also revealed an increase in rat plasma β-endorphin levels after haloperidol treatment (Höllt & Bergmann 1982).

These facts made us question whether the increase in the content of endorphins produced by the blocking of dopaminergic receptors by haloperidol could be due to an increase in the biosynthesis of endorphins or possibly to a blocking of the enzymes that metabolize these peptides. Previous work has shown different findings; while some authors have demonstrated that haloperidol produces an increase in the biosynthesis (Hong et al 1979), in-vitro work suggests that haloperidol and other dopaminergic blockers are potent inhibitors of the enzymatic breakdown of endorphins (Jakubovic 1982).

In our studies, the fact that after the administration of cycloheximide the inhibitory response was significantly modified in those animals treated with haloperidol, but not in those treated with saline, indicates that the increase in the release of endorphins produced by haloperidol would be due to an increase in the biosynthesis of endogenous opiate peptides produced by this drug. Recent experiments provide strong evidence that in the rat, chronic haloperidol treatment increases the messenger RNA activity coding for pro-opiocortin in the intermediate pituitary (Höllt 1981), which is in favour of the hypothesis that the blocking of dopaminergic receptors increases the synthesis of endorphins.

No alteration of the opiate receptor is produced after treating guinea-pigs with haloperidol or cycloheximide. The dose-response curves performed with met-enkephalin and reversal of its effect by naloxone in haloperidol- or cycloheximide-treated guinea-pigs do not show any significant variation compared with a control group.

In conclusion, our results provide evidence that interruption of the dopaminergic neuronal transmission in guinea-pig ileum myenteric plexus by haloperidol, produces changes in the biosynthesis and release of endorphins at this level, which could suggest that dopamine would play an important role in regulating the biosynthesis and release of endorphinergic systems in the guinea-pig ileum myenteric plexus.

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