Inhibition of dopamine biosynthesis by gonadotropin-releasing hormone in rat

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There is evidence that the neuroendocrine system can be modulated by endogenous opioid peptides (for a review see ref. 1). Recently Rotsztejn *et al.*[2] have ruled out a direct effect of Met-enkephalin on release of gonadotropin-releasing hormone (GnRH). Instead they postulated that Met-enkephalin inhibits the secretion of dopamine from dopaminergic neurones thereby reducing the dopamine-stimulated release of GnRH from the hypothalamus. We report here evidence that GnRH can itself supress dopamine synthesis in the rat. The fact that the dopamine neurone and GnRH-secreting cell are adjacent to each other[3] suggests a feedback mechanism of regulating GnRH release.

Sprague–Dawley rats (180–230 g) were killed by decapitation. The corpus striatum was dissected on ice and homogenized in 10 vols 0.32 M sucrose using a Teflon pestle tissue homogenizer. After centrifugation at 1,000*g* for 15 min, 50 μl aliquots of the synaptosome-containing supernatant were incubated with 150 μl of physiological medium containing (mM): 125 NaCl; 1.48 $CaCl_2$; 4.8 KCl; 2.5 $MgSO_4$; 22 NaH_2PO_4; 10 $NaHCO_3$; 16 glucose, to give a final *p*H of 6.6 after equilibration with 95% O_2–5% CO_2 at 37 °C. [1-^{14}C]tyrosine (specific activity 50 mCi mmol^{-1}) at a concentration of 10 μM was added to the preparation, together with bacitracin (10^{-5} M) to reduce the degradation of polypeptides. GnRH, Met-enkephalin or naloxone were added to the incubation medium with 10 μl 0.1 M phosphorous buffer *p*H 6.6 as carrier; controls recieved the buffer alone. The method used to determine dopamine synthesis was modified from that of Weiner[4] and Kuzcenski[5], who measured the release of $^{14}CO_2$ from [1-^{14}C]tyrosine. We simplified the incubation medium to mimic the cerebrospinal fluid and used a respirometer to supply oxygen and allow continuous measurement of the $^{14}CO_2$ output from the tissue[6]. Addition of iodotyrosine (5×10^{-4} M) to the incubation medium inhibits >90% of the $^{14}CO_2$ release[7].

Dopamine formation was calculated when the $^{14}CO_2$ output had reached a steady state, that is, after 50 min incubation. The rate of dopamine synthesis in 10 preparations was 0.2 ± 0.02 mol mg^{-1} min^{-1} (mean ± s.d.).

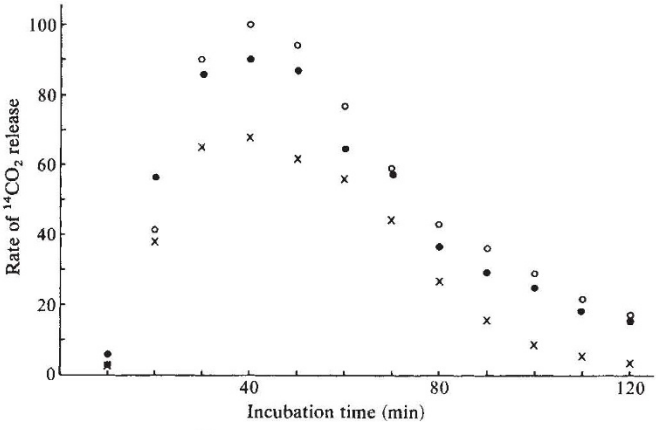

Fig. 1 Rate of $^{14}CO_2$ release from labelled tyrosine plotted against incubation time. The exponential increase in rate before 50 min of incubation is from isotopic equilibrium[6]. The rate of $^{14}CO_2$ release maintained steady-state values between 50 and 120 min of incubation. GnRH (5×10^{-6} M) added to the preparation (×) gave ~30% inhibition of dopamine synthesis. Naloxone at 5×10^{-6} M together with GnRH blocked 70% of this inhibition (●). ○, Control.

Met-enkephalin (10^{-5} M) added to the incubation mixture reduced $^{14}CO_2$ release by ~40%. Although some of this inhibition could be due to the degradation of the enkephalin releasing unlabelled tyrosine, we showed by kinetic studies using added unlabelled tyrosine that >10% of this inhibition was a direct effect. Other opioid δ-receptor drugs, for example, D-Ala^2-Met-enkephalin and Metkephamid[8] at 10^{-5} M also inhibited dopamine synthesis by ~10% and 20% respectively and this inhibition was not blocked by naloxone. In contrast, the 11% inhibition of dopamine synthesis by sufentany[1] (10^{-5} M), a μ-receptor drug, was almost totally blocked by naloxone.

When GnRH (5×10^{-6} M) was incubated with the synaptosomal preparation, the $^{14}CO_2$ release was reduced to 73.4 ± 6.6% of the normal rate. (The relative amounts of $^{14}CO_2$ released from the added tyrosine are shown in Fig. 1.) Naloxone (5×10^{-6} M) together with GnRH (5×10^{-6} M) blocked ~70% of the inhibitory effect. The normal $^{14}CO_2$ release was reduced by GnRH at 1×10^{-6} M and 5×10^{-7} M to 83.1 ± 3.8% and 89.9 ± 2.7% respectively. GnRH at a concentration of $<1\times10^{-8}$ M had no significant effect.

These data show that GnRH and δ- and μ-receptor opioids all have similar inhibitory effects on dopamine synthesis. We therefore postulate that GnRH exerts a negative feedback action on dopaminergic neurones, that is, GnRH inhibits it own release by inhibiting dopamine synthesis.

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Glutamate regulates adenylate cyclase and guanylate cyclase activities in an isolated membrane preparation from insect muscle

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It is generally believed that glutamate serves as a neurotransmitter at many vertebrate and invertebrate synapses. There is, however, little information concerning the possible involvement of cyclic nucleotides as intracellular second messengers for any of the postsynaptic actions of glutamate. Direct activation of adenylate cyclase has been reported for several neurotransmitters and neuromodulators[1], but no such effect has been reported for glutamate. Moreover, although several neurotransmitters including glutamate have been shown to increase cyclic GMP levels in intact preparations of nerve and muscle tissue[2,3] apparently by promoting Ca^{2+} entry into the cells, no neurotransmitter has been shown to activate guanylate cyclase in a cell-free preparation. L-Glutamate is the prime candidate for the neurotransmitter at the excitatory neuromuscular synapses in insects[4]. Moreover, recent studies have shown that glutamate application raises both cyclic AMP[5] and cyclic GMP levels (P.M.C., unpublished observations) in insect muscle. To determine the mechanism underlying these increases, we have studied the effect of glutamate in a membrane fraction prepared from this muscle, and report here that the neurotransmitter activates both adenylate cyclase and guanylate cyclase in the membrane preparation, and that the activation by glutamate of guanylate cyclase is calcium independent.

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