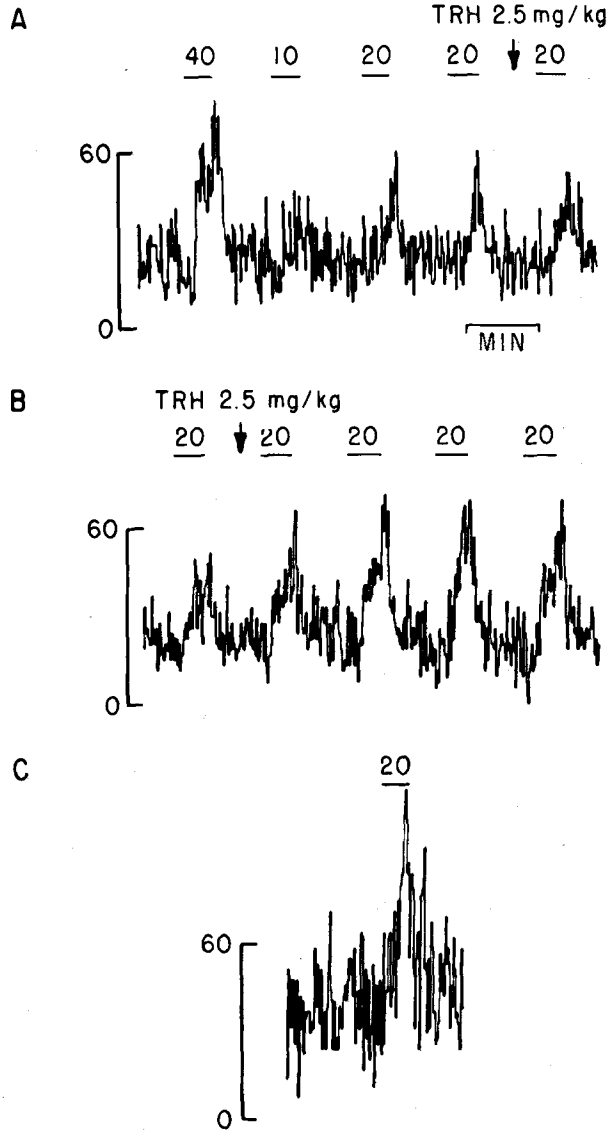
Intravenous thyrotropin releasing hormone (TRH) enhances the excitatory actions of acetylcholine (ACh) on rat cortical neurons

G. G. Yarbrough and D. K. Singh

Merck Institute for Therapeutic Research, West Point (Pennsylvania 19486, USA), 4 July 1977

Summary. I.v. administered TRH (1.25–10 mg/kg) enhanced the excitatory actions of iontophoretically applied ACh on spontaneously active cerebral cortical neurons of pentobarbital-anaesthetized rats. These observations are consistent with the hypothesis of a cholinergic-link in the anti-anaesthetic actions of exogenously administered TRH.

The ability of TRH to offset the narcosis produced by barbiturates in laboratory animals is antagonized by cholinergic muscarinic antagonists[1,2]. Moreover, iontophoretically applied TRH enhances the excitatory actions of ACh and carbachol (but not glutamate) on spontaneously active cerebral cortical neurons of pentobarbital anaesthetized rats and it was suggested that this action of TRH may have some relevance to the anti-anaesthetic properties of the peptide[3]. In order to further evaluate this hypothesis it appeared necessary to determine if systemically administered TRH, at doses comparable to those which antagonize barbiturate-induced loss of the righting reflex[1], would also enhance the excitatory actions of ACh on rat cortical neurons.



Effect of i.v. TRH to enhance the response of a cortical neuron to iontophoretically applied ACh. Spikes sec^{-1} are represented on the ordinate. The lines above the traces indicate periods of ACh ejection and the numbers refer to amounts of current in nanoamperes. A and B are continuous. C was recorded 30 min later.

These studies were conducted on 8 female Charles River rats (180–210 g) anaesthetized with 50 mg/kg i.p. of pentobarbital Na. The animals were paralyzed with 15 mg/kg of Flaxedil (initially, with supplements when required) and artificially respirated through a tracheal cannula. A cannula for drug injection was placed in the jugular vein. The animals were placed in a stereotaxic frame and body temperature was maintained at 37 °C through a rectal probe connected to a heating pad through a feedback device. A small burr hole was drilled over the somatosensory cortex and a glass micropipette (tip diameter 4–8 μm) containing 2 M NaCl in the center barrel (for neuronal recording) and 1 outer barrel (for automatic current balancing) and 0.5 M ACh in another outer barrel was placed 800–1430 μm below the cortical surface. The surface of the skull and the wound were covered with 4% agar to prevent drying. Only 1 spontaneously active cortical neuron was examined in each rat to avoid residual drug effects. TRH was dissolved in 0.9% saline at various concentrations for i.v. injection.

Cumulative i.v. doses of 1.25 to 10 mg/kg of TRH enhanced with a latency of 0.5–3 min the excitatory actions of iontophoretically applied ACh in 7 of 8 rats tested (i.e. 7 of 8 neurons). An example of this effect is shown in the figure which is a rate meter tracing of a spontaneously active neuron located at a depth of 946 μm. It can be seen that after a cumulative dose of 5 mg/kg the response of the neuron to 20 nA of ACh is clearly enhanced, approaching that seen in the pre-drug period to 40 nA of ACh. The record in C, taken 30 min after B, illustrates an elevated spontaneous firing rate and an even greater response to ACh. Since the magnitude of the responses to ACh both before and after TRH varied considerably among the experimental preparations, it appears unjustified to draw any conclusions regarding potency or minimal effective doses. Nonetheless, the doses of TRH employed in these studies are certainly within the range necessary to elicit behavioral (i.e. anti-anaesthetic) responses in intact animals.

These data indicate that systemically administered TRH in relevant pharmacological doses can, like iontophoretically applied TRH, enhance the excitatory actions of ACh on pentobarbital-anaesthetized rat cortical neurons. Since there is evidence in the literature to suggest that anaesthetics reduce neuronal excitatory responses to ACh[4,5], these findings are consistent with the postulate of a cholinergic participation in the anti-anaesthetic properties of this neurally active peptide.

1 G. R. Breese, J. M. Cott, B. R. Cooper, A. J. Prange, M. A. Lipton and N. P. Plotnikoff, J. Pharmacol. exp. Ther. *193*, 11 (1975).
2 A. Horita, M. A. Carino and R. M. Chestnut, Psychopharmacologia *49*, 57 (1976).
3 G. G. Yarbrough, Nature *263*, 523 (1976).
4 K. Krnjevic and J. W. Phillis, J. Physiol. (Lond.) *166*, 328 (1963).
5 F. E. Bloom, E. Costa and G. C. Salmoiraghi, J. Pharmacol. exp. Ther. *150*, 244 (1965).