damaged DNA. Such damage was confirmed by autoradiography of cells exposed to [$^3$H]thymidine, which indicated that DNA repair had been initiated by ultrasound (*10*). Both single-strand scission and repair mechanisms have been implicated in the basic mechanism of SCE production, and it is not surprising that ultrasound increased the frequency of SCE in the present studies. The magnitude of the effect was small, but the increase was detectable in every experiment.

Our results with cells exposed to ultrasound in vitro cannot be directly extrapolated to the clinical situation where the ultrasound probe is in constant motion and no one region is continuously exposed to the beam. Most clinical examinations of the fetus are of short duration and the surrounding maternal tissues attenuate the ultrasonic beam. However, our findings suggest that ultrasound may not be entirely innocuous.

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# Thyrotropin-Releasing Hormone Selectively Depresses Glutamate Excitation of Cerebral Cortical Neurons

Abstract. *The microiontophoretic application of thyrotropin-releasing hormone causes a selective reduction in neuronal excitation evoked by* L-*glutamate but not by acetylcholine in rat cerebral cortex. Thyrotropin-releasing hormone has no influence on the activity of acetylcholinesterase or on choline uptake and release from cerebral synaptosomes. This evidence for a selective interaction between a centrally acting peptide and an excitatory amino acid neurotransmitter may indicate a specific locus of thyrotropin-releasing hormone action at glutamate-activated receptor sites.*

Thyrotropin-releasing hormone (TRH) can directly influence central nervous system (CNS) function, independent of its role as a releasing hormone (*1*). The proposal that this direct CNS effect results from the action of TRH as a neurotransmitter or neuromodulator has been supported by several observations: (i) TRH is present in the brain of several vertebrate and nonvertebrate species, including those that lack a thyroid gland (*2*); (ii) TRH is localized within neurons (*3*); (iii) TRH is concentrated within, and can be released from, preparations of synaptosomes (*4, 5*); (iv) high-affinity stereoselective TRH binding sites are present within the brain (*6*); and (v) exogenous applications of TRH can alter central neuronal excitability (*7, 8*). In previous studies (*8*), we indicated that microiontophoretic application of TRH is associated with a decrease in spontaneous or glutamate-evoked activity in several brain regions—an observation that has prompted further examination of the specificity of the action of TRH. We now report that in the cerebral cortex of the rat, TRH selectively antagonizes neuronal excitation evoked by L-glutamate. TRH has less of an effect on L-aspartate-evoked excitation and no significant effect on acetylcholine-evoked excitation.

Extracellular recordings were obtained from neurons in the sensorimotor cortex of male Sprague-Dawley rats anesthetized with pentobarbital (35 mg/kg, intraperitoneally) or urethane (1.25 g/kg, intraperitoneally). Conventional amplification methods were used to record extracellular activity from micropipettes filled with 2*M* NaCl that were rigidly attached to multibarreled micropipettes filled with solutions for testing by microiontophoresis (*9*). TRH from different sources and in different concentrations and solvents (*10*) was applied as a cation; currents ranged up to a maximum of 100 nA. Additional channels contained sodium chloride (0.2*M*) for use as a current control and sodium chloride (2.0*M*) for use in an automatic current balancing circuit (*11*).

Experiments on 42 neurons examined the influence of TRH on the excitatory actions of two putative amino acid neurotransmitters, L-glutamate and L-aspartate. In 35 of these neurons, the application of TRH was associated with a rapid, readily reversible reduction in amino acid-induced excitation, without any significant alteration in spike amplitude or shape. When applied with stronger ejection currents, TRH appeared to diminish excitation evoked by both amino acids; however, when applied with weaker ejection currents, the action of TRH was more specific and most pronounced in the glutamate-evoked responses (Fig. 1). This action of TRH on amino acid-evoked excitability was considerably more prominent than its relatively weak depressant action on the spontaneous activity of 3 of 26 neurons tested.

To examine the specificity of this depressant action, TRH was applied during excitations induced by acetylcholine on cholinoceptive cortical neurons (*12*).

Table 1. Thyrotropin-releasing hormone and acetylcholinesterase activity, uptake of [$^{14}$C]choline in pellet, and $K^+$-evoked release of $^3$H from [$^3$H]choline. The number of experiments is given in parentheses.

| TRH ($\mu$g/ml) | AChE activity* ($\mu$mole/hour-mg protein) | Uptake of [$^{14}$C]choline† (nmole/5 min-mg protein) | $K^+$-evoked release of $^3$H from [$^3$H]choline |
|---|---|---|---|
| Control | 3.09 ± 0.68 (6) | 1.05 ± 0.05 (9) | 2505 ± 475 (3) |
| 1 | 3.20 ± 0.62 (6) | | |
| 5 | | 1.03 ± 0.09 (3) | |
| 10 | 2.90 ± 0.70 (6) | | |
| 20 | | 1.02 ± 0.02 (3) | |
| 50 | 3.00 ± 0.50 (6) | | 2510 ± 610 (3) |

*Values represent average activity (mean ± standard error) of three assays from the same $P_2$ preparation. †Protein concentration, 1 to 3 mg/ml.


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TRH, applied with currents up to 100 nA for periods as long as 5 minutes, failed to produce any significant change in the acetylcholine-evoked activity of 47 neurons (Fig. 2A). When acetylcholine and glutamate were alternately applied to 11 neurons, the failure of TRH to modify acetylcholine-induced excitation contrasted sharply with the depressant effect of TRH on glutamate-evoked excitation (Fig. 2, B and C). This differential effect was not observed when the same 11 cells were tested with γ-aminobutyric acid (GABA); GABA depressed the excitation evoked by acetylcholine and glutamate. In addition, the effects of GABA (but not of TRH) were reversibly antagonized by picrotoxin and bicuculline [compare (*8*)]. Thus, it would appear that TRH exerts its effects predominantly (and perhaps specifically) on glutamate-activated receptors. Acetylcholine also depressed the excitability of 12 cortical neurons (*12*). TRH did not modify acetylcholine's depressant effects, just as it did not modify acetylcholine's excitatory actions. These observations, therefore, support the interpretation that TRH directly and selectively alters cortical neuron excitability evoked by acidic amino acids (especially glutamate). TRH also alters spontaneous neuronal activity, but this direct effect of TRH is comparatively weak and is less commonly observed. The consistent pattern in the recorded responses suggests that these data do not result from indirect or secondary effects of drug interaction with adjacent neurons.

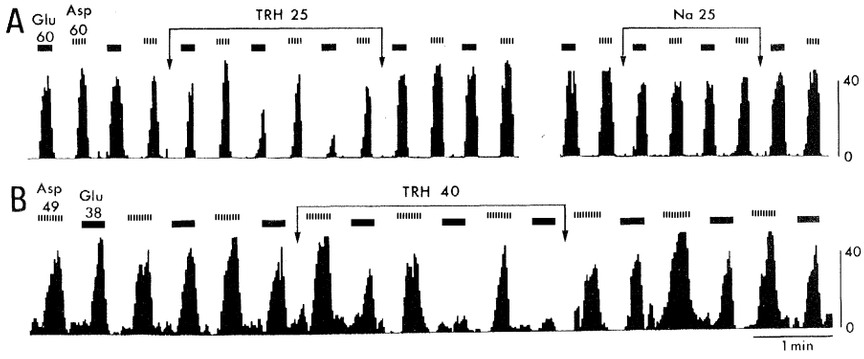


Fig. 1. (A and B) Ratemeter records from two different cortical neurons whose activity was enhanced by intermittent microiontophoretic applications of L-aspartate (*Asp*) and L-glutamate (*Glu*). Each deflection represents the action potential frequency per second. The bars represent the current (in nanoamperes) used to apply each agent. The scales on the far right correspond to an action potential frequency of 40 Hz. During the application of TRH there is a gradual, readily reversible decrease in responses evoked by glutamate (but not by aspartate)—a decrease that does not occur during the application of sodium ions (positive current).

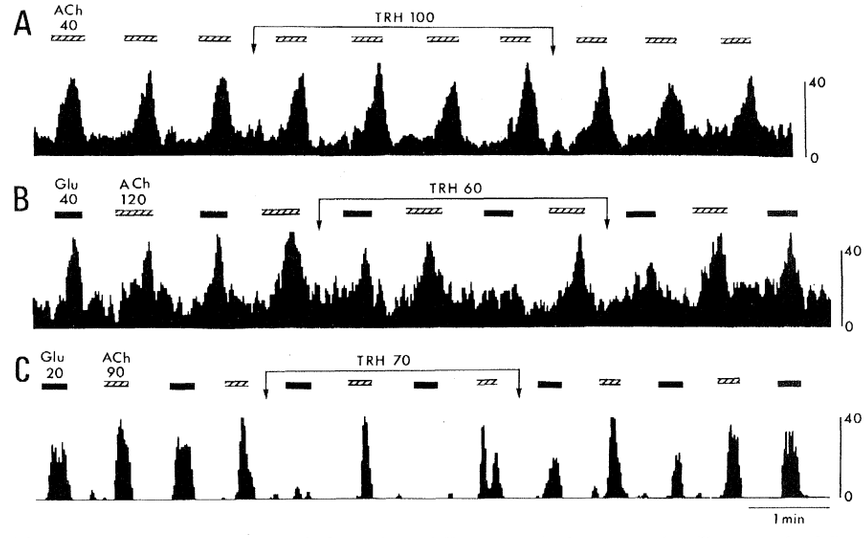


Fig. 2. (A to C) Ratemeter records from three different cortical neurons that illustrate the failure of TRH to alter excitations induced by intermittent microiontophoretic application of acetylcholine. However, TRH continues to have a selective and reversible depressant action on glutamate-evoked excitations (records B and C). (For a detailed explanation, see Fig. 1.)

Recently, Yarbrough (*13*) reported that systemic or microiontophoretic application of TRH (and of certain TRH analogs) is associated with a selective potentiation of the excitatory actions of acetylcholine on sensitive cortical neurons. However, this and other (*14*) electrophysiological studies have failed to confirm Yarbrough's results. Moreover, the 3-methylhistidine analog of TRH, which is more potent than TRH in the release of thyroid-stimulating hormone (*15*), mimics the selective depressant effect of TRH on activity evoked by glutamate (but not by acetylcholine).

We ran biochemical tests (Table 1) to seek additional evidence of a possible TRH-acetylcholine interaction. In synaptosomes prepared from isolated guinea pig and rat cortices (*16*), TRH had no significant influence on acetylcholinesterase activity (*17*), the uptake of labeled choline (*18*), or choline release (*19*). Thus, both our electrophysiological and biochemical data argue against a significant effect of TRH on the actions of acetylcholine in cerebral cortex. More investigations are required to clarify the nature of a previously described (*20*) interaction between cholinergic systems in the brain and TRH-induced antagonism of hypothermia and of ethanol and barbiturate narcosis.

The evidence that an endogenous peptide can selectively alter the activity of one endogenous neurotransmitter substance suggests that TRH may have a specific role in modifying postsynaptic actions at glutamate-activated receptor sites on cortical neurons.

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10. The TRH (supplied by Abbott, Peninsula, and Beckman laboratories) was dissolved as a 10 m*M* solution in distilled water or as a 50 m*M* solution in 165 m*M* sodium chloride (*p*H 7.0).
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19. Release studies involved several steps. First, the $P_2$ suspension was labeled with radioactive choline as described in (*18*), except that the final choline concentration was 1 μ*M* and the incubation was for 15 minutes at 37°C. Then the [$^3$H]choline-labeled $P_2$ suspension was separated from the supernatant by centrifugation, washed with buffer, and resuspended in KR phosphate buffer. Equal portions of [$^3$H]choline-labeled $P_2$ suspension were pipetted into KR buffer and concentrated potassium buffer (final KCl concentration, 40 m*M*). Finally, suspensions in both KR and potassium buffers were incubated at 37°C for 10 minutes and centrifuged, after which the radioactivity in the supernatant was counted.
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# Acridine Araphanes: A New Class of Probe Molecules for Biological Systems

Abstract. *The bis-acridine ring system forms the basis for new biophysical probes of novel stereochemistry. Spectral data indicate that certain alkylene bridged bis-9-aminoacridines have a parallel plane conformation of predictable interplane distance. The parallel plane conformation is independent of solvent and thus is different from nucleic acid systems. This stable conformation allows these compounds to be used as sensitive "rulers" for describing binding site geometry in cholinergic enzymes and in the delineation of the mechanism of allosteric control in acetylcholinesterase.*

As a part of a study of cholinergic neural systems, a series of alkylene bridged bis-9-aminoacridines were prepared by the method of Chen *et al.* (*1*), and were found to exhibit major spectral anomalies, which were related to bridge length (*n*) (Fig. 1). They suggested a conformation change from an open (coplanar) conformation ($n > 4$) to a parallel plane conformation when $n = 2, 3$ (or 4).

Parallel plane conformations are known to exist when aromatic molecules form concentration dimers, stacks, or excimers in concentrated solution, or in oriented polymers such as DNA, base pair systems, and in bridged bis-adenines in water solution (*2–4*). In these cases, the parallel plane conformation is either concentration dependent or stable only in the presence of water, and disappears on dilution. DNA base pairs and bridged bis-adenines are denatured or lose spectral effects (hypochromatism), when dissolved in ethanol (*4*).

Spectral data indicated that the parallel plane conformation in alkylene bridged bis-9-aminoacridines was independent of the nature of the solvent or dilution. Nuclear magnetic resonance (NMR) spectra indicated a dynamic rather than a rigid conformation. The diversity and predictability of dynamic conformations, independent of solvent, suggest that this series of singly bridged bis-acridines are new probes for biological systems. We report data from four spectral methods and uses of a parallel plane, molecular probe in the study of the active site stereochemistry of acetylcholinesterase (E.C. 3.1.1.7).

The bis-acridine series have only a single bridge; they have the potential for a preferred parallel plane conformation. They exist in that conformation in aqueous or organic solvents ($10^{-6}M$) only when the total interplanar forces are sufficient to constrain the movement of the two rings in relation to each other. The parallel plane conformation was identified as follows.

In water and methanol, molecules having a parallel plane conformation manifest three distinct differences in their ultraviolet absorption spectra from the spectra of their parent 9-alkylaminoacridine. (i) The complex three-peak structure at 410 nm (n-$\pi^*$) is lost to a broad single peak in the related parallel plane structure. (ii) The strongly absorbant $\pi$–$\pi^*$ transition peak at 268 nm shows a progressively increasing blue shift with decreasing bridge length. (iii) The shift of the $\pi$–$\pi^*$ absorption is accompanied by a progressive decrease in extinction coefficient (Fig. 2). Similar progressive abnormalities are noted in the fluorescence spectra of these compounds. (i) The emission, which consists of a lone, broad peak at 486 nm, is diminished and replaced by a series of two peaks at a shorter wavelength in the bridged bis-acridines, which exist in a parallel plane conformation. A red shift occurs in the emission maximum as solvent polarity decreases. (ii) There is also a decrease in quantum yield as the bridge length shortens between the two rings, stemming from the transannular electronic effects that occur when two aromatic moieties are in close proximity and in parallel planes.

Absorption and fluorescence spectra of parallel plane molecules in highly polar solvents correspond to the spectra of simple 9-alkylaminoacridines in nonpolar solvents. This effect indicates that bis-acridines in which the rings are parallel have a hydrophobic environment between the rings, in aqueous solution (*5*).

The spectral data described above are consistent with a transition from a parallel plane conformation when $n = 2$ or 3 to a coplanar conformation when $n$ is 6 or greater, with a time resolved average parallel conformation observed when $n = 4$.

Proton NMR spectral data (deuterated dimethylsulfoxide) were consistent with conclusions derived from ultraviolet and fluorescence data when the number of bridge carbons is two. When $n = 3$, the increased distance limited NMR-resolvable effects. However, the bis-acridines identified as open (coplanar) conforma-


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