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Effect of Thyrotropin Releasing Hormone on Heart Rate and Visual Evoked Potentials in Rats

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Synopsis

The effect of injection of 0.3 μg of thyrotropin releasing hormone (TRH) into the third ventricle on heart rate and photically evoked potentials in the midbrain reticular formation in urethane-anesthetized rats was studied. TRH increased the heart rate and amplitude of the visual evoked potentials within a few minutes following injection. The intraventricular injections of LHRH, constituent amino acids of TRH and intraperitoneal TSH did not exert any effect. This indicates that TRH is effective in the central nerveous system in inducing tachycardia in the rat.

Several investigations from the group of Plotnikoff, Prange and others have indicated that the psychopharmacological effect of TRH may not be mediated by the pituitary-thyroid axis but direct on the central nervous system (CNS) (Plotnikoff, *et al.*, 1974; Prange *et al.*, 1974). These studies stem from the clinical work demonstrating that triiodothyronine will potentiate the antidepressant effects of tricyclic drugs in women (see Prange, *et al.*, 1974). It should be noted that the effects of TRH and thyroid hormones in CNS seemed to be in the same direction. It is well known that thyroid hormones augment heart rate, but the mechanism of this action still remains unclear (Freedberg and Hamolsky, 1974; Bray and Jacobs, 1974). It was demonstrated by Shimada and Oshima (1973) of this laboratory that thyroxine (T4) increased the heart rate in the chicken following a short latent period. This was observed only when the treatment was applied to these birds while they were exposed to darkness when the vagal tone was presumably elevated. Then the results were considered to suggest that T4 was effective on CNS in controlling heart rate. On the other hand, Hine *et al.*, (1973) reported that intravenous injection of TRH caused tachycardia in the conscious dog. The present author has noted that the consistent bradycardia results in the chicken by raising the ambient temperature from 25 to 37°C in 30 min (unpublished observation). Since TRH synthesis and/or release are sensitive to the change in ambient temperature (Moll *et al.*, 1972; Reichlin and Mitnik, 1973; Knigge and Joseph, 1974) TRH may take a role in the regulation of heart rate affected by the temperature change. In consequence of these considerations, we set out to explore the possible TRH-induced alterations of neurophysiologic events which may result in a change in heart rate. The present paper describes the effect of injection of TRH into the third ventricle on heart rate in rats and concomitant change in photically evoked

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responses in the midbrain reticular formation as an indication of direct and broad effect of TRH in CNS.

Materials and Methods

Experiments were carried out on male albino rats of Wistar-Imamichi strain, weighing between 350 and 500 g. The animals were maintained in a temperature (22°C) and light (14 hr light) controlled room with food and water supplied ad libitum. Experimental recordings were made between 45 and 120 min after anesthetizing the animal by intraperitoneal (ip) injection of urethane (120 mg/100 g). Electrocardiogram (EKG) was recorded continuously by means of a polygraphic recorder with the electrodes inserted bilaterally on the breast. The surgical procedure for electroencephalographic (EEG) recordings consisted in exposing the skull and stereotaxically introducing stainless steel bipolar electrodes, with a inter-tip distance of 1.0 mm, into the midbrain reticular formation (A, 2.0 mm; L, 1.5 mm; D, 1.0 mm in the stereotaxic atlas of König and Klippel, 1963), and securing the electrodes with dental resin. EEG recording was started about 20 min after urethane injection. Background EEG profiles somewhat differed from rat to rat but were generally dominated with the wave of 6–7 Hz at the amplitude of 20–50 μV during the experimental period. Large spikes (sometimes over 100 μV) appeared at the frequency of about 1/min on the first 20 min recordings but the spikes dissipated to about 1/5 min after 45 min following urethane injection. Thus the visual evoked response experiments were carried out between 50 and 100 min after urethane injection. Evoked potentials were produced by delivering photic stimuli by a photostimulator (Nihon Koden, Co.) with a xenon lamp (mean intensity, 7.5 lux at a distance of 30 cm in 10 f/sec) placed 30 cm in front of the rats. Thirty two flashes of 0.1 msec duration with 1.0 sec interval were delivered and the electrical activities were amplified by a preamplifier (120S, Sanei Sokki, Co.) and displayed on an oscilloscope of Signal Processor (7S06, Saneki Sokki, Co.), the results were added and the curve was plotted on a recorder. After observing the stable EEG and EKG recordings for 10 min, the test substance dissolved in 3 μl saline was injected into the third ventricle at the level of nucleus anterior hypothalami by means of a microsyringe introduced stereotaxically. The amount of 0.3 μg of synthetic TRH or luteinizing hormone releasing hormone (LHRH) (Protein Research Foundation, Osaka), was injected as a standard dose. In the controls, 3 μl of saline or 0.3 μg of equimolar mixture of glutamic acid, histidine and proline was injected into the ventricle followed by an intraperitoneal injection of thyrotropin (TSH, Thyroper, Armour, 100 mu dissolved in 0.1 ml saline).

Results

In the preliminary experiment it was ascertained that the heart rate was fairly stable for 45 to 120 min after the ip injection of urethane. The intraventricular injection of 3 μl saline did not exert any effect on the heart rate. Fig. 1 illustrates the change in heart rate following the intraventricular injection of TRH (0.3 μg). Within one min following TRH injection the acceleration of heart rate was observable, the rate increased linearly to 7–8 min and leveled off thereafter. On the other hand, the intraventricular injection of equimolar mixture of proline, glutamic acid and histidine followed by ip TSH did not elicit any change in heart rate. The alteration of EEG responses evoked by photic stimulation in the midbrain reticular formation following TRH injection was also studied. The recorded patterns and amplitude of evoked responses varied more or less among the rats or locations of the electrodes. But in most recordings it was observed that the initial large positive wave with the latency of 12–16 msec was followed by a negative wave with the peak latency of 36–60 msec (Fig. 2.). Also several less pronounced waves appeared with longer latencies. In some recordings sharp deflections of positive or negative direction either preceeded or were superimposed on the major waves (Fig. 3.)

The intraventricular injection of saline or amino acids mixture and ip TSH did not produce any observable change in latencies or amplitude of evoked responses. Intraventricular TRH also did not affect the latencies of the evoked responses but did consistently produce the increase of the amplitude within 2 min following injection (Fig. 2 and 3.). Intraventricular LHRH seemed to affect somewhat the minor de-

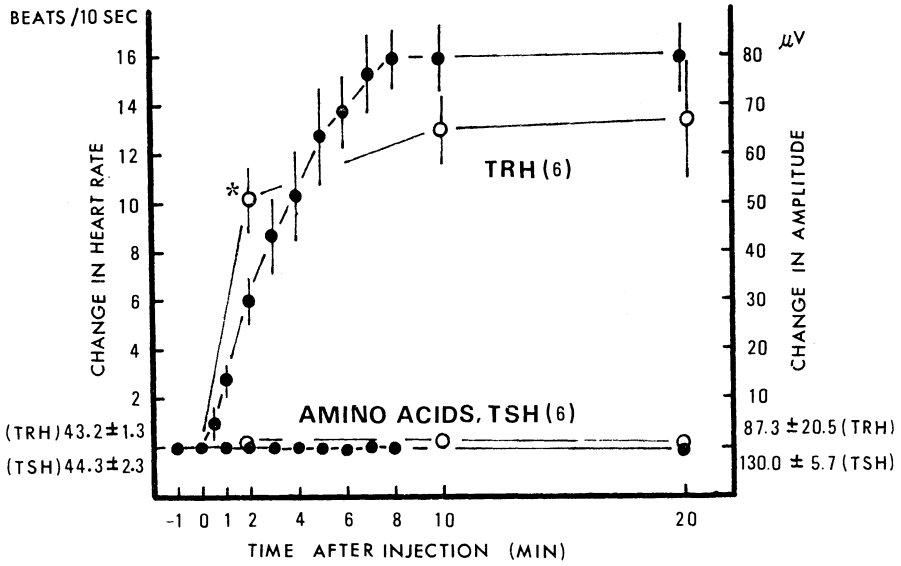

Fig. 1. Change in heart rate and peak-to-peak amplitude in evoked potentials in the midbrain reticular formation. The value just before the injection in each animal was used as its own control. Mean±S.E. of the control was denoted on the left (heart rate) or right (amplitude) side at 0 level of the figure with the respective treatment in parentheses. Open circles, average changes in amplitude. Closed circles, average changes in heart rate. Vertical bars, standard errors. Number in parentheses, number of animals. The asterisk denotes that the change is statistically significant at the 1% level thereafter.

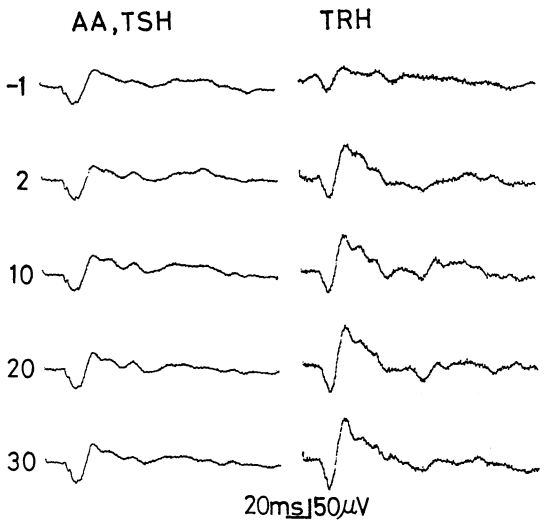

Fig. 2. Photically evoked potentials in the midbrain reticular formation before, and 2, 10, 20 and 30 min after the injection of TRH or amino acids mixture plus TSH.

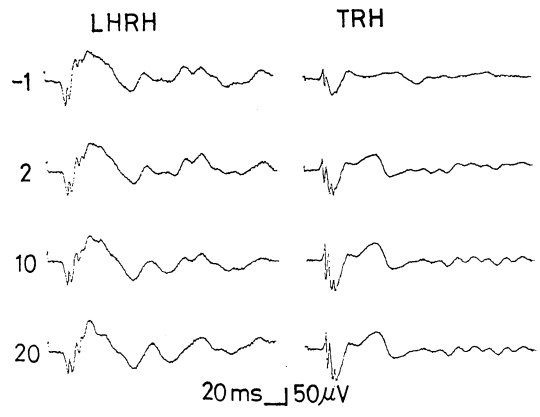

Fig. 3. Photically evoked potentials in the midbrain reticular formation before, and 2, 10, and 20 min after the TRH and LHRH injection.

flections but the latencies and amplitude of the major waves were not altered up to 20 min following injection (Fig. 3.). A summary of the data as to the effect of TRH on the pead-to-peak voltage of the major waves, using each animal as its own control, is illustrated in Fig. 1. More than 60% increase in amplitude is obvious within 2 min and at 10 min the amplitude reaches the highest level.

Discussion

Knigge and Joseph (1974) demonstrated the presence of TRH in cerebrospinal fluid of the third ventricle of rats and suggested that TRH is transported through the third ventricle. Several investigations have also reported that significant quantities of TRH are present in the various hypothalamic and extrahypothalamic regions surrounding the third ventricle (Oliver *et al.*, 1974; Brownstein *et al.*, 1974; Krulich *et al.*, 1974; Winokur and Utiger, 1974). It seems, thus, reasonable to use the intraventricular route for exploring the effect of TRH in CNS.

Hine *et al.*, (1973) reported that tachycardia was produced by intravenous injection of 100 μg/kg of TRH in the conscious dog, but the time course of the effect was not described. In the present report it was demonstrated that intraventricular TRH elicits an increase in heart rate essentially without any latent period which is inevitably required in the effect of thyroid hormone treatment. Failure of TSH to induce the tachycardia in 20 min following treatment may allow us to conclude that the effect of TRH is not mediated by pituitary-thyroid axis even if time required for diffusion of TSH into circulation is taken into account. Several psychopharmacological investigations in animals have suggested that TRH may have a direct central effect in sympathetic activation (Prange *et al.*, 1974); (Breese *et al.*, 1974); (Plotnikoff *et al.*, 1974). In many of these experiments the used route of administration of TRH was intraperitoneal, intravenous or oral and the dosages were in the range of 0.1 to 20 mg/kg. In the dopa potentiation test in mice and rats the effective dose of TRH were over 0.1 mg/kg (Plotnikoff *et al.*, 1974). Thus, the effectiveness of 0.3 μg TRH on the acceleration of the heart rate in the present experiment, suggests a direct effect on CNS rather than one affecting the peripheral system. Segal and Mandell (1974) demonstrated that intraventricular infusion of TRH at the rate of 90 nmoles/hr in rat increased the spontaneous activity.

In the reticular formation its complex polysynaptic pathway abolishes the modality specificity but a summated excitation of a series of neurons brings into an active state by integration of the local potentials. Thus it was assumed that the effect of TRH might be appreciable in the response of the reticular formation if TRH has broad effects in CNS.

The alteration in the amplitude of evoked potentials in the midbrain reticular formation following intraventricular TRH injection provides another evidence of the central action of TRH. The fact that LHRH failed to elicit an effect suggests that the TRH effect is not merely caused by a change in physicochemical nature of cerebrospinal fluid, resulting from an addition of peptides. At present, it is impossible to delineate the precise mechanism of this TRH action on CNS. However, it is plausible that TRH affects the multisynaptic systems in the brain stem and increases the brain excitability as was indicated in the effect of adrenocortical hormone on the electrical activity in the brain (Feldman and Dafny, 1970). If the present finding of the increase in the amplitude of evoked potentials in the midbrain reticular formation is assumed to reflect the augmented excitability of the ascending reticular activating system (RAS), this may result in the change in heart rate

since RAS activityis known to influence widely on the cardiovascular organization.

Twelve years ago, Short *et al.*, (1964) demonstrated that triiodothyronine (T3) treatment increased the amplitude of evoked potentials of the cortex and reticular formation of the cat. In their experiment, however, a significant effect was observable 2 hours after the intravenous injection of T3 and the magnitude of the change in the reticular formation was less than 20%. It should be noted that the effect of TRH in the present experiment is more rapid and more marked; the increase in the amplitude was 60% of the control and reached to 75% of the highest level within 2 min.

Thus the present results show that TRH rapidly elicits CNS responses (change in heart rate and evoked potentials) which are also induced by thyroid hormone treatment. It should be remembered here that thyroid hormones block the TRH release but stimulate TRH synthesis (Reichlin and Mitnik, 1973). Thyroid hormones may augment the CNS level of TRH which, in turn, affects CNS activity, resulting in tachycardia as described in the present study.

Whether this relationship between thyroid hormones and TRH in controlling CNS activity is concerned with the phylogenetic development of TRH-TSH-thyroid axis in homeotherms is a very interesting problem. Knigge and Joseph (1974) have presented the data suggesting that TRH distribution between cerebrospinal fluid and median eminence is altered by T4 treatment.

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ADDENDUM

After submission of this paper, note was taken of a paper by R. Mueller, G. R. Breese, A. J. Prange, J. K. Lewis, H. Morrison and W. T. Mckinney in Pharmacology Biochemistry and Behavior. 4. 109. 1976. These authors observed a rapid increase in heart rate by the intravenous injection of TRH in the pentobarbital anesthetized monkey.

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