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Motoneurone excitability after administration of a thyrotrophin releasing hormone analogue

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1 Recordings have been made of motoneurone field potentials at vertebral level L1 to stimulation of the ventral root, in rats anaesthetized with urethane.

2 Injection of thyrotrophin releasing hormone (TRH) analogue RX77368 (2 mg kg^{-1}) produced an increase in the amplitude of the field potential within 1 min of injection, reaching peak amplitude of 51–69% above control values 6–12 min after injection; the duration of the elevation was 30–65 min.

3 Arguments are presented which show that this effect is compatible with an RX77368-induced depolarization of the motoneurone, allowing the antidromic volley to invade more cell bodies.

Introduction

Previous investigations have shown that administration of thyrotrophin releasing hormone (TRH) or its analogues produces changes in motor activity, e.g. wet dog shaking (Webster, Griffiths & Slater, 1982) and can potentiate the motor effects of other neurotransmitters, e.g. 5-hydroxytryptamine (5-HT)-induced hyperactivity (Green & Grahame-Smith, 1974). Ono & Fukuda (1982) demonstrated in spinal rats that TRH analogues produced increases in the monosynaptic reflex without alterations in the dorsal root reflex or the dorsal root resting potential and concluded that there was a postsynaptic effect involving depolarization of the membrane of the lower motoneurone. The present experiments have been performed to determine whether depolarization of the motoneurone membrane does occur after systemic administration of a TRH analogue, by recording the field potentials of motoneurone pools to antidromic stimulation (Barakan, Downman & Eccles, 1949) before and after administration of the TRH analogue, RX77368.

Methods

Anaesthesia

Ten female rats (Sheffield strain) in the weight range 190–210 g were used. Each was anaesthetized with urethane (25% in 0.9% saline i.p.) in amounts sufficient to abolish hind limb withdrawal to a strong pinch (1.25–1.5 g kg^{-1}).

Preparation

A tail vein was cannulated to permit subsequent injections and a laminectomy performed (L2-S1). A paraffin pool was made over the exposed cord utilising the cut skin edges, and the vertebral column was clamped rigidly proximal and distal to the exposed section. The dura mater was then removed. Body temperature was measured by rectal thermometer and maintained within 0.5 °C of 37 °C.

Stimulation and recording

Bipolar steel electrodes were used to apply electrical stimuli (at a rate of 0.25 Hz) to ventral root L5, the root being severed distal to the electrodes. Extracellular recordings were made of the field potentials from groups of motoneurones (Barakan *et al.*, 1949). Stimulus strength was adjusted to give a suprathreshold submaximal response. Recordings were made using 3 M saline filled glass microelectrodes. Since antidromic stimulation results in activity in a number of structures (e.g. medullated and non-medullated sections of efferents, Renshaw cells), care was taken to identify positively the motoneurone response by the latency, (> 0.5 ms) rise time (= or > 0.3 ms), duration of waveform (= or > 1 ms) and lack of repetitive discharge. Also care was taken to make sure the response would fractionate smoothly with changes in stimulus strength thus ensuring recording was from populations of, rather than single motoneurones, since this affects the in-

terpretation of the antidromic response. The antidromic potentials were amplified and displayed on a Tektronix R510 3N storage oscilloscope and also stored on tape (Maxell U.D. C90 on an Alba 3300 tape deck) or averaged with a Neurolog NL750 averager and plotted on a Bryans 2800 recorder.

Drugs

Thyrotrophin releasing hormone analogue (*p*Glu-His-(3,3'-dimethyl-Pro NH_2) (RX77368) kindly supplied by Reckitt and Colman, Hull, Yorkshire) was given as an intravenous injection of 2 mg kg^{-1} dissolved in 0.9% saline at a concentration of 2 mg ml^{-1}. The delivery of the injection took 30–60s.

Analysis

The amplitude of the negative component of the motoneurone field potential was measured at 1 min intervals from the averaged response to the first eight stimuli in each minute period. Measurements were taken from 10 min before RX77368 injection to 65 min after. The same measurements were also taken over this period in 5 control animals after drug vehicle injection. Pre-injection amplitude was considered as a 100% response, subsequent responses expressed accordingly. Tests of significance were performed using a single sample, single tail, paired *t* test.

Results

Antidromic responses have been recorded in 10 rats to stimulation of the ventral root L5.

Location of responses

Field potentials were recorded at vertebral level L1 at depths ranging from 1100–1500 μm (mean= 1290 μm). Measurements taken from histological material showed that this region contains motoneurones; the position agrees with the data of Iles & Nicolopoulos (1981).

TRH analogue and antidromic response

In each of the 5 experimental animals the amplitude of the negative component of the field potential started to rise within 1 min of injection of RX77368 (Figure 1), reaching a peak 51–69% (mean =60.8%, s.e. =±3.22 n =5, $P<0.005$) above control values 6–12 min (mean= 9.6) after injection. The amplitude remained elevated above control values for 30–65 min. The time course of the response is shown in Figures 2 and 3). In the 5 controls no changes in the amplitude were observed in the hour following injection of 0.2 ml saline. To assist with the interpretation of the effect of RX77368 on the antidromic response, the effect of a dorsal root conditioning stimulus on the antidromic

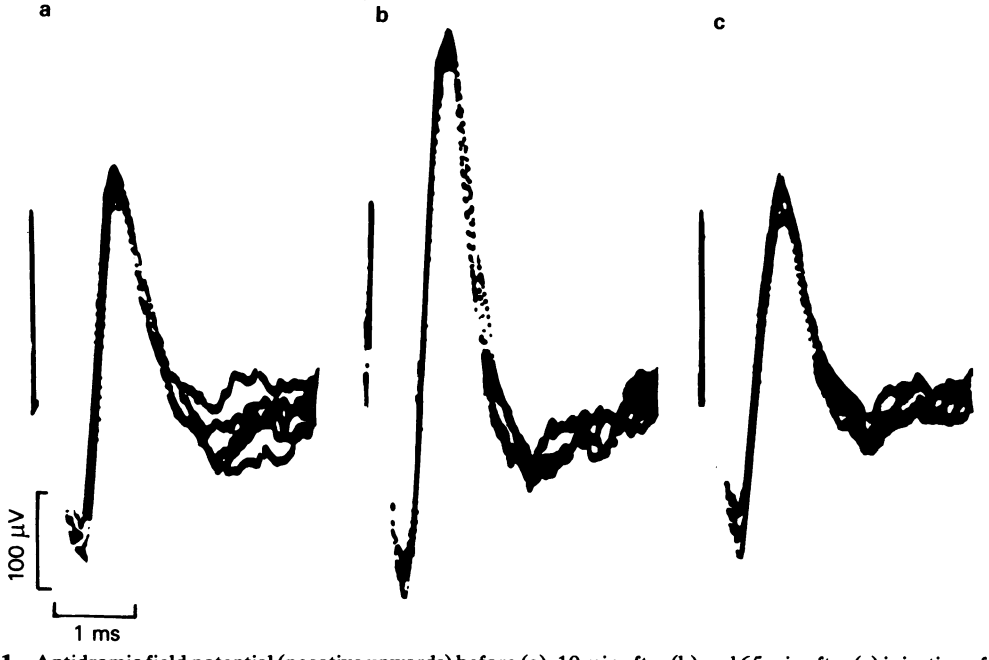

Figure 1 Antidromic field potential (negative upwards) before (a), 10 min after (b) and 65 min after (c) injection of RX77368. Each trace consists of 5 consecutive sweeps superimposed.

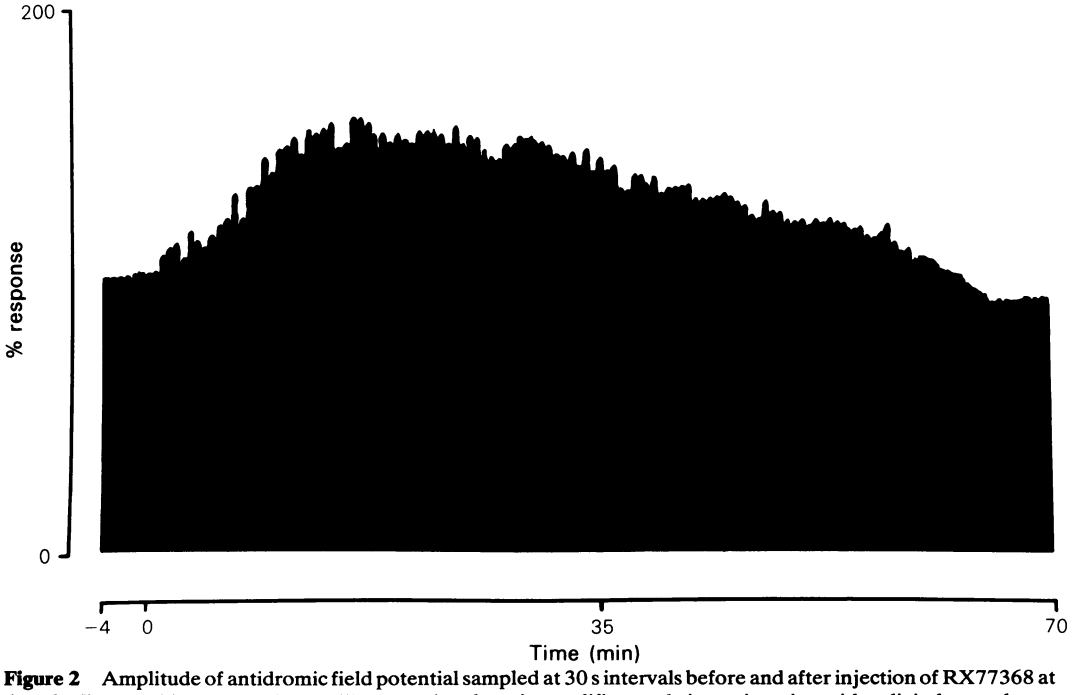

Figure 2 Amplitude of antidromic field potential sampled at 30 s intervals before and after injection of RX77368 at time 0. Figure achieved by using oscilloscope time base in amplifier mode in conjunction with a digital to analogue converter. Each response appears therefore as a vertical deflection.

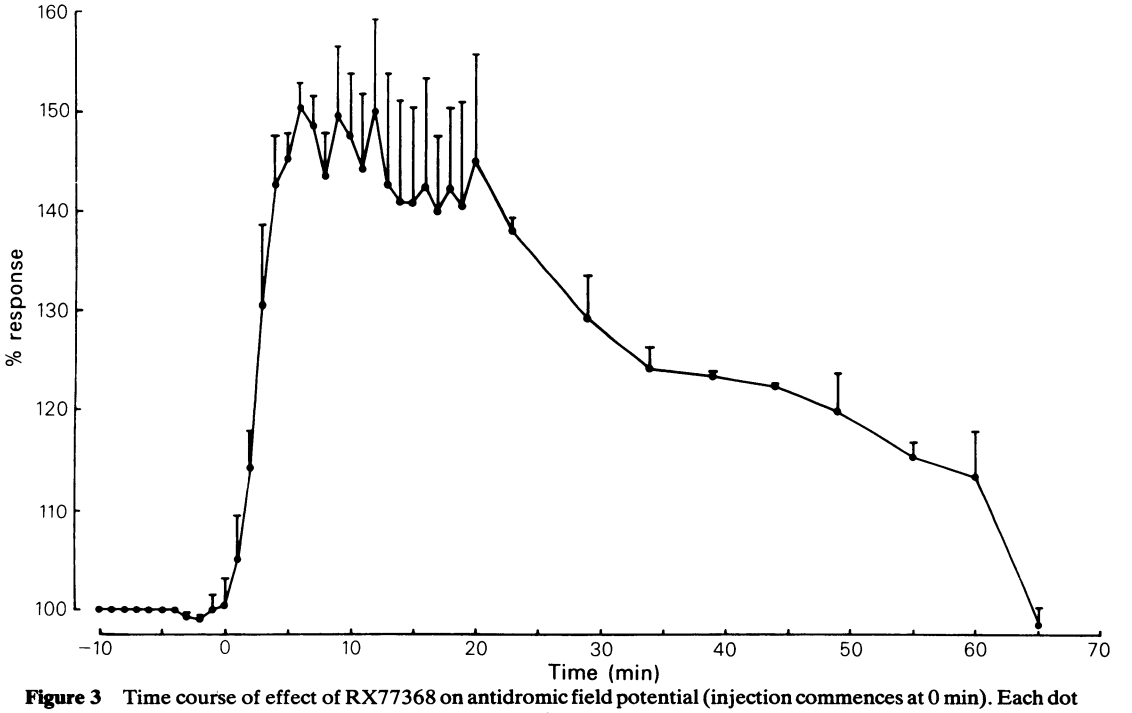

Figure 3 Time course of effect of RX77368 on antidromic field potential (injection commences at 0 min). Each dot is the mean % response (with s.e. shown by vertical lines) with respect to pre-injection responses (=100%) from 5 experiments. Irregular appearance of plateau response caused by variation in time to reach peak in the different experiments.

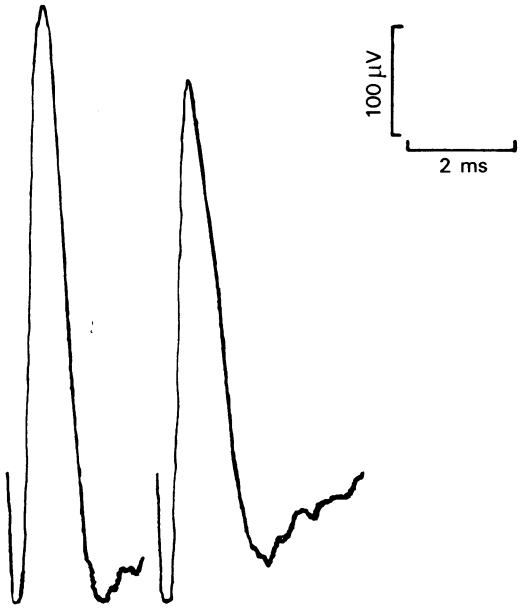

Figure 4 Antidromic field potential (negative upwards) conditioned by dorsal root stimulation of lowest threshold fibres (left) and unconditioned (right).

response was observed in two of the control animals. In both cases the conditioning stimulus caused an increase in the antidromic response (Figure 4).

Discussion

It has been shown that systemic injection of 2 mg kg^{-1} of the TRH analogue, RX77368, produces large increases of approx. 51–69% in the motoneurone field potential evoked by antidromic stimulation, the peak increase being around 10 min after injection and the duration of the effect some 30–65 min. Since a depolarizing conditioning stimulus also caused an increase in the response to the subsequent antidromic test stimulus, it is concluded that an increase in the antidromic response occurs under conditions of motoneurone depolarization. Similar conclusions have been reached with other depolarizing agents such as glutamate (Nicoll, 1977) and potassium (Parry & Roberts, 1980). It is concluded therefore that the increase in amplitude to antidromic stimulation after administration of RX77368 was caused by depolarization of the motoneurone membrane, allowing the antidromic volley to advance into more somas than previously. Iontophoretic studies showing that TRH depolarizes frog lower motoneurones (Nicoll, 1977) would support these conclusions. The present experiments therefore support the conclusion of Ono *et al.* (1982) to the extent that TRH analogue RX77368 does produce depolarization of the lower motoneurone. Thus, when the phasic excitatory input is evoked to produce the monosynaptic reflex, this input (which may itself be qualitatively and quantitatively different from the control) will encounter a motoneurone pool in a partially depolarized state. The mode of action of RX77368 in producing this depolarization of the lower motoneurone, therefore, remains to be determined. Segmental effects such as tonic changes in excitatory input from Ia afferents could be envisaged. Since spinal animals were not used in the present experiments, activation of descending systems cannot be ruled out. Marsden, Bennett & Irons (1982) have shown that TRH is found in high concentrations in the ventral horn of the rat spinal cord stored within the same bulbospinal terminals as 5-HT. Since 5-HT also causes motoneurone depolarization (McCall & Aghajanian, 1979), the functional integration of these two putative transmitters on motoneurone activity clearly requires investigation.

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