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THE EFFECTS OF TRIMETHADIONE ON PENTETRAZOL-INDUCED DISCHARGES OF PRIMARY MUSCLE SPINDLE AFFERENTS FROM THE HIND LIMB OF THE RAT

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1 Recordings have been made from primary muscle spindle afferents in split dorsal root filaments of rats anaesthetized with urethane.

2 Injection of pentetrazol (PTZ, 10 mg/kg) produced elevated discharge in the afferents with intact efferents without any changes in tension or electrical activity of the muscle of origin.

3 This elevated discharge was found to contain elements of both γd and γs activation.

4 Trimethadione (100 mg/kg), itself produced a transient increase in discharge and effectively suppressed PTZ-induced discharges of the afferents for at least 2 h.

5 These results are discussed in the context of established effects on sensory and motor systems.

Introduction

In numerous studies, trimethadione (3,5,5-trimethyl-2,4-dioxooxazolidine, TMD), a drug which has been used in the treatment of petit mal epilepsy, has been shown to antagonize the effects of pentetrazol (PTZ, pentamethylenetetrazole), e.g. on the discharge of vestibular neurones (Kirsten & Schoener, 1972), on spinal reflexes (Esplin & Curto, 1957), on the metabolism of 5-hydroxytryptamine (5-HT) (Diaz, 1974). Angel & Clarke (1980b) demonstrated that PTZ produces elevated activity in primary muscle spindle afferents by effects on both the γd and γs elements of the fusimotor system. The present work has been concerned with discovering whether the antagonistic actions of PTZ and TMD can be demonstrated on the discharge of muscle spindle afferents.

Methods

Anaesthesia

A total of 24 female albino rats (Sheffield strain) in the weight range 195 to 205 g have been used. Each rat was anaesthetized with an intraperitoneal injection of urethane (25% in 0.9% aqueous saline in 1.0 ml per 200 g body weight primary dose). This dose was normally sufficient to abolish withdrawal of the hindlimb to a strong pinch. If not, more urethane was administered up to a total dose of 1.5 g/kg.

Preparation

Procedures for dissection and fixation of the animal and for recording, identification and classification of muscle spindle afferents have been fully described in a previous paper (Angel & Clarke, 1980a).

Recording

Recordings were made from split dorsal root filaments with a steel microelectrode, the tip of which had been bent over to form a small hook. In some experiments, bipolar steel electrodes were used to check whether any muscle potential developed at the doses of PTZ used. With the amounts used, there was no detectable EMG activity. Muscle tension was also monitored. Recordings from dorsal root and muscle were fed through an F.E.T. follower, amplified and displayed on an oscilloscope, (Tetronix type R5103N) and simultaneously recorded on magnetic tape (Scotch Dynarange C-90 cassettes on an Akai GXC-40D cassette recorder).

Stimulation

A short piece of surgical silk was attached to the distal tendon of the muscle, the other end of the silk being attached to a lever mounted on a modified EEG motor. The pen motor was connected to the output of a waveform generator which allowed ramp and hold stretches (ramp velocity 0.25 mm/s, amplitude 0 to 0.75 mm, each held for 3 s and delivered at a rate of one per 9 s) or sinusoidal stretches (1 Hz; amplitude 0 to 0.75 mm p to p) to be applied to the muscle. Experiments in which movement of the lever was monitored by means of an optical wedge and photo transistor showed that its motion followed the

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output from the waveform generator in all important respects.

Analysis

Analysis of discharge frequencies was approached in two ways. Discharges from unstretched endings were summed over 1 s periods and displayed on a chart recorder (Bryans 28000). Discharges from stretched endings were translated into instantaneous frequencies by leading the pulse train into a frequency to voltage converter involving the following steps; level comparator, integration, log ratio amp., inversion, antilog amp. The output voltage could be calibrated by means of a pulse generator.

Identification

Positive spindle identification was made by observing the cessation of discharge during semi-isotonic contraction of the muscle of origin from a starting length sufficient to produce afferent discharge (Fulton & Pi Sũner, 1928; Matthews, 1933).

The criterion for division into primaries and secondaries was based upon dynamic index measurements. That is, the decrease in frequency which occurs in the first 500 ms after completion of a ramp and hold stretch (Harvey & Matthews, 1961; Crowe & Matthews, 1964). These were superimposed on previously determined curves for primary and secondary muscle spindle afferents (Angel & Clarke, 1980a).

Pentetrazol

PTZ (pentamethylenetetrazole, Sigma Chemical Co., St. Louis, U.S.A.) was administered intravenously via a tail vein cannula. The drug was dissolved in 0.9% w/v NaCl solution (saline) at a concentration of 10 mg/ml, injection duration <2 s and washed in with a further 0.1 ml saline. Most of the measurements were the responses to subconvulsive doses of 10 mg/kg PTZ. This dose did not cause detectable changes in either electromyographic activity or tension in the muscle of origin of the spindle.

Trimethadione

Trimethadione (3,5,5-trimethyl-2,4-dioxooxazolidine, supplied by Abbott Laboratories Ltd., Queensborough, Kent) was administered intravenously via a tail vein cannula. It was dissolved in saline at a concentration of 20 mg/ml as a single dose of 100 mg/kg, injection duration <20 s and washed in with a further 0.1 ml saline.

Measurements

Frequencygrams of the instantaneous frequency of firing to stretch before and after PTZ were constructed. Criteria were adopted to identify γd and γs activity.

Ramp and hold stretches were employed to allow changes in dynamic index to be measured (Harvey & Matthews, 1961; Brown & Matthews, 1966). An increase in dynamic index was taken to indicate predominant γd activity and a decrease predominant γs activity. With large amplitude sinusoidal stretches the maximum instantaneous frequency per cycle and the tendency to fall silent on release were measured (Emonet-Dénand, Laporte, Matthews & Petit, 1977). An increased frequency to the lengthening part of the cycle (LF) with a small or absent firing on release (RF) was taken to indicate a predominant γd effect. A large increase in firing on release (RF $>$ 10% LF) was taken as significant γs activity.

Results

A total of ten primary muscle spindle afferents have been isolated from split filaments in the dorsal roots of 10 anaesthetized rats with intact efferents. These originated from spindles in the plantar and dorsiflexors of the ankle and toes.

Effect of pentetrazol on muscle activity

In a number of experiments electrical activity and/or tension was simultaneously monitored from the contralateral leg muscles. In no instance was there any measureable change in either muscle tension or electrical activity at doses of PTZ of up to 20 mg/kg.

Effect of pentetrazol on de-efferented endings

The effect of PTZ on the discharge frequency of 14 acutely de-efferented muscle spindles was obtained. In no instance was the pre-injection steady firing level elevated by 10 mg/kg PTZ given intravenously.

Effect of pentetrazol on resting frequency of endings with intact efferents

Prior to PTZ injection, 8 of the 10 afferents had a resting discharge of zero. The other two had discharges of 8 and 18 imp/s. Within 10 s of an intravenous injection of PTZ (10 mg/kg) the discharge frequency began to rise in each of the afferents. This would plateau over the next few seconds and control values were obtained within 1 min of injection. The smallest frequency increase was 15 imp/s, and the largest 85 imp/s (mean increase 38.9 imp/s, s.e. $= \pm 7.5$, $n = 10$). The effect of PTZ injection on the discharge of a primary spindle afferent is shown in Figures 1 and 2.

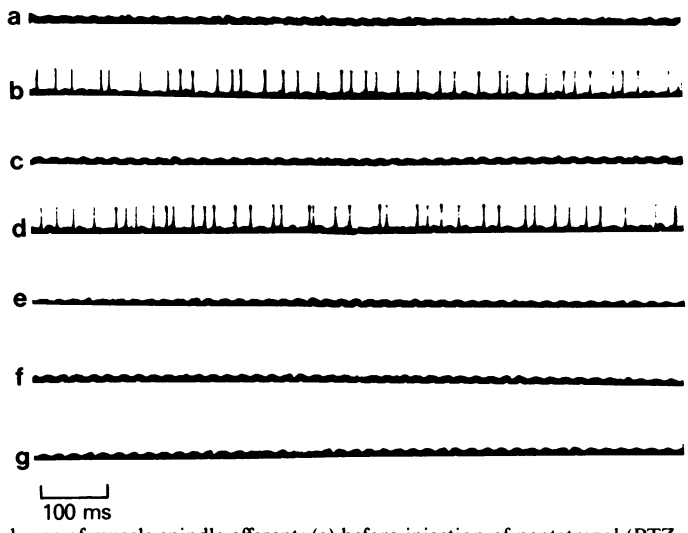

Figure 1 Resting discharge of muscle spindle afferent: (a) before injection of pentetrazol (PTZ, 10 mg/kg); (b) 10 s after injection of PTZ; (c) 80 s after injection of PTZ; (d) 20 s after injection of trimethadione (100 mg/kg); (e) 40 s after injection of trimethadione; (f) further injection of PTZ 3 min after injection of trimethadione; (g) further injection of PTZ 2 h after injection of trimethadione. Time marker on abscissa scale is 100 ms.

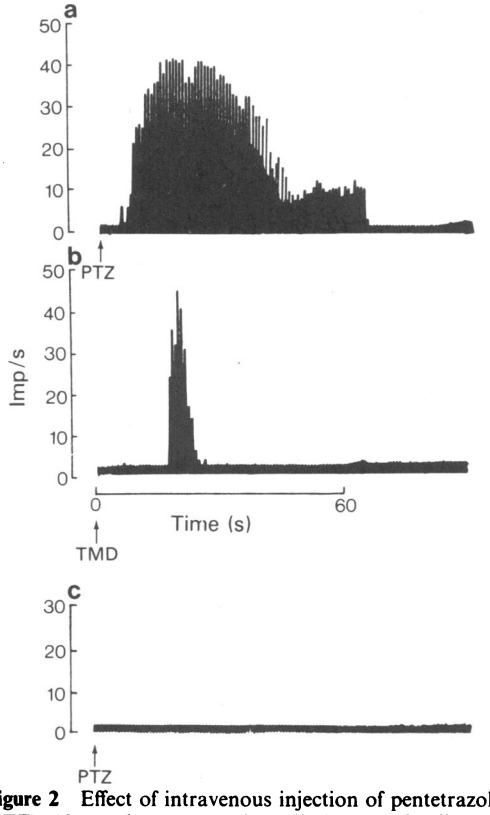

Figure 2 Effect of intravenous injection of pentetrazol (PTZ) 10 mg/kg, on resting discharge of afferent summed over 1 s periods, before (a) and 3 min after (c), injection of trimethadione (TMD), 100 mg/kg (b).

Effect of pentetrazol on response to stretching

When the endings were subjected to ramp stretches before and at various times after PTZ there were some periods during the course of drug action on a single afferent in which there was an increased dynamic index with no firing on release, implying γd fusimotor activity. At other points there was a reduction in dynamic index with marked firing on release, implying γs activity. The typical effect on a primary is seen in Figure 3. Experiments with sinusoidal stretching confirmed these observations.

Effect of trimethadione on resting frequency

In 5 out of the 10 afferents, an intravenous injection of TMD (100 mg/kg) produced a brief elevation in discharge frequency lasting approx 10 s. In some cases the peak frequency attained was almost as great as PTZ injection although the duration of the effect was much less and the latency to onset longer. This effect on one of the endings is shown in Figures 1 and 2.

Effect of trimethadione pentetrazol-induced discharge

Three minutes after injection of TMD (100 mg/kg) the animal was given an intravenous injection of PTZ (10 mg/kg). In 9 of the afferents there was no increase in discharge frequency above the pre-injection level. In the tenth there was a much smaller increase in discharge frequency than attained previously (from zero to 16 imp/s compared with from zero to 68 imp/s).

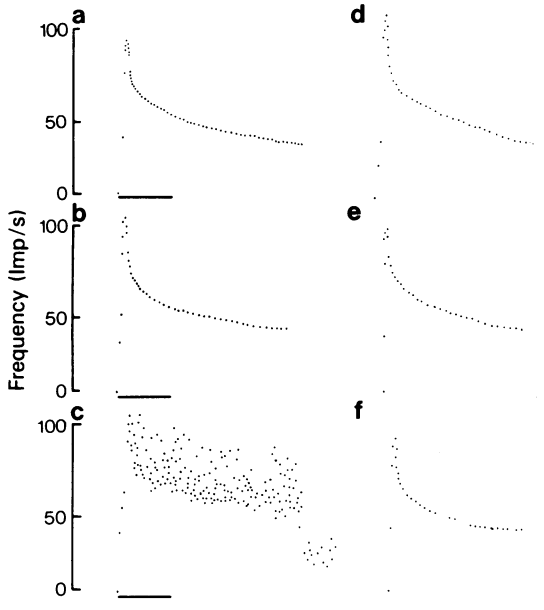

Figure 3 Response of a primary spindle afferent to a ramp stretch of 5 mm/s of its muscle of origin before (a), 10 s after (b), 20 s after (c), 30 s after (d), 40 s after (e) and 50 s after (f) an intravenous injection of pentetrazol of 10 mg/kg. Horizontal marker is 1 s.

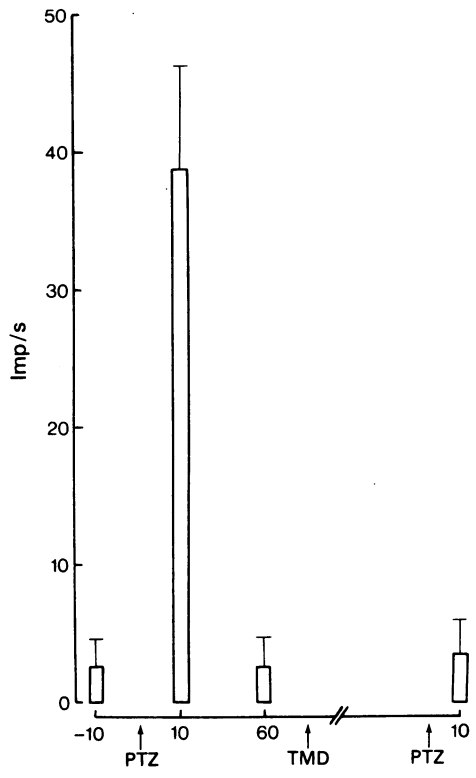

Figure 4 Effect of pentetrazol (PTZ) injection, 10 mg/kg, on mean discharge frequency (Imp/s, $n = 10$; vertical lines show s.e.) of whole group of 10 afferents before and after injection of trimethadione 100 mg/kg (TMD). Numbers on abscissa scale are the times (s) related to the appropriate injection point.

The effect of PTZ on discharge frequency before and after TMD is shown in Figures 1, 2 and 4. The suppression was still present when tested up to 2 h after TMD injection (Figure 1).

Discussion

The present experiments have shown that TMD effectively suppresses discharges in primary muscle spindle afferents induced by subconvulsive doses of PTZ. Although it has been demonstrated that PTZ elevates activity in both γd and γs elements of the fusimotor system (Angel & Clarke, 1980b), TMD is not necessarily acting at the same points in suppressing this activity. In addition, low levels of γd activity would not necessarily be expressed as an elevated afferent discharge of the passive ending and it is therefore not possible to say from the present experiments whether TMD interferes with the expression of this activity.

Opposing effects of PTZ and TMD have been observed in 5-hydroxytryptaminergic pathways. Diaz (1974) observed PTZ-induced decreases and TMD-induced increases in 5-HT turnover in whole rat brains. However, intravenous injections of 5-hydroxytryptophan (5-HTP, a precursor of 5-HT), in spinal animals, has been found to increase the tonic vibration reflex (vibration of amplitude 50 to 100 μm, frequency 50 to 200 Hz, applied to muscle) by facilitating transmission from group Ia afferents of primary spindle endings to α motoneurones (Ellaway & Trott, 1975). Also in spinal animals, 5-HTP has been found to increase static fusimotor discharge (Ahlman, Grillner & Udo, 1971). This evidence, while possibly offering some explanation for the transient elevation of afferent discharge after TMD in the present experiments, does not help in the interpretation of the suppression by TMD of PTZ-induced discharges.

Perhaps there are important effects on sensory systems which lead ultimately to the activation or inactivation of the descending motor systems. For instance, PTZ is known to affect primary afferent depolarization (Benoist, Besson & Boissier, 1974) and antagonize γ-aminobutyric acid (GABA)-induced depolarization of afferents in cuneate nucleus (Simmonds, 1980) indicative of a reduction in presynaptic inhibition, while TMD has been shown to increase presynaptic inhibition (Miyahara, Esplin & Zablocka, 1966). PTZ

has veratrine-like effects on excitable tissues (Eyzaguirre & Lilienthal, 1949) and decreases synaptic recovery time whereas TMD increases synaptic recovery time (Esplin & Curto, 1957). TMD is known to affect sensory processes by its effects on colour vision and visual acuity (Sloan & Gilger, 1947). Descending 5-hydroxytryptaminergic pathways have been shown to inhibit interneurones in the spinal cord driven by noxious stimuli (Randić & Yu, 1975). PTZ has predictable effects on the discharge of units in the thalamic reticular formation, an area known to affect information transfer through the thalamic sensory relay nucleus (Angel and Clarke, unpublished observations). Further work is required to elucidate how the balance between sensory input and motor output is altered by convulsants and anti-epileptics.

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