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The effect of EU4093 (azumolene sodium) on the contraction of intrafusal muscle in the soleus muscle of the anaesthetized rat

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1 EU4093 (azumolene sodium) is a direct acting, skeletal muscle relaxant with structural similarities to dantrolene sodium in that the *para*-nitro phenyl group of dantrolene sodium is replaced by a *para*-bromo phenyl group.

2 The effect of EU4093 on the twitch of the intact rat soleus preparation is nearly maximal at a dose of 20 mg kg^{-1}. This dose of EU4093 reduces the amplitude of the twitch to 31.9% of the control value. By comparison, dantrolene sodium reduced the twitch amplitude to 31.3% of the control value at a dose of 5 mg kg^{-1}.

3 The contraction of intrafusal muscle as measured by the response of spindle afferent discharge was also reduced by EU4093.

4 The most significant difference between the effect of EU4093 and dantrolene sodium on the contraction of intrafusal muscle was that EU4093 had an appreciable depressant action at high stimulation frequencies at which dantrolene sodium had only a minimal relaxant effect on the intrafusal muscle contraction.

5 The significance of this difference in the actions of EU4093 and dantrolene sodium is discussed in terms of the possible effect of the drug in the intact animal and human patient. It is concluded that, because the discharge frequency is not likely to move into the high frequency range at which dantrolene sodium and EU4093 have significantly different effects, the overall effect of these two drugs is likely to be similar.

Introduction

Abnormal skeletal muscle contraction may be controlled pharmacologically by drugs that act either directly on muscle or indirectly on the central nervous control of muscle. Dantrolene sodium is an example of a drug which acts directly upon skeletal muscle at the level of excitation-contraction coupling (Ellis & Bryant, 1972). Recently derivatives of dantrolene sodium have been synthesized (White *et al.*, 1987). Several of these compounds have also been shown to have a direct muscle relaxant effect (White *et al.*, 1987; Pong *et al.*, 1987). We describe here the effect of one of these compounds (EU4093, azumolene sodium, see Figure 1) on the contraction of intrafusal muscle in anaesthetized rats.

Muscle spindle afferents have an excitatory action upon synergist motoneurones. Any relaxant effect of a drug on the intrafusal muscle will thus reduce the excitatory input onto the alpha motoneurones. Data on the action of the drug on the contraction of intrafusal muscle are essential therefore in order to understand the mode of action of the drug in the intact patient.

The most striking feature of the action of dantrolene sodium is the relationship between the depression of muscle contraction and stimulation frequency for both extrafusal (Bowman *et al.*, 1979; Leslie &

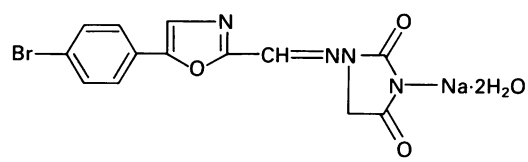

Figure 1 The structure of EU4093 (azumolene sodium). The full chemical name of this compound is 1-[[[5-(4-bromophenyl)-2-oxazolyl]methylene]amino] - 2,4-imidazolidinedione sodium salt dihydrate.

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Part, 1981a) and intrafusal (Leslie & Part, 1981b) muscle. If the stimulation frequency is sufficiently great, the muscle is spared the relaxant effect of the drug. In this present work we have investigated the effect of EU4093 on the contraction of intrafusal muscle at a range of stimulation frequencies. The methods used were similar to those used previously for investigation of the action of dantrolene sodium (Leslie & Part, 1981b).

Methods

The experiments were carried out on a total of 16 female Sprague Dawley rats, 300–400 g body weight. Anaesthesia was induced with trichlorethylene vapour and maintained by intraperitoneal injection of urethane, 1500 mg kg^{-1} body weight. Supplementary injections were given as necessary.

The soleus muscle was used for these experiments. The use of this preparation for spindle experiments has been described previously (Andrew *et al.*, 1978) and is outlined in Figure 2. Briefly, the muscle was prepared for stimulation of the muscle nerve and the distal tendon tied either to a tension transducer

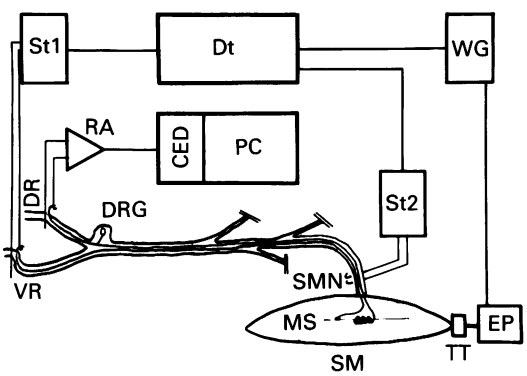

Figure 2 Simplified diagram of the experimental arrangement used for the recording of intrafusal muscle contraction. Stimulation of the ventral root (VR) filament and soleus muscle nerve (SMN) is by means of two stimulators (St1 and St2, respectively) both controlled by a Digitimer (Dt) control system (Digitimer Ltd). The digitimer is also used to control a waveform generator (LF141, Servomex Ltd) which in turn controls an electromagnetic puller (EP). The tension in the soleus muscle (SM) is recorded with a tension transducer (TT). The afferent discharge of a muscle spindle (MS) is recorded in a dorsal root (DR) filament with a recording amplifier (RA) and hence passed via a CED1401 computer interface (CED) to a PC-AT compatible computer (PC). In making this preparation a gamma fibre has been selected that activates the muscle spindle primary afferent previously isolated.

(Devices, 2ST02, 0–1 kg range) or to the arm of an electromagnetic puller with a tension transducer mounted upon it. Single spindle afferent nerve fibres were isolated in dorsal root filaments and single gamma nerve fibres active to the same spindle in ventral root filaments. The response of the spindle afferent to stimulation of the gamma efferent was recorded with the muscle held at constant length. Action potential discharges were displayed as instantaneous frequencies. Permanent records of the frequency displays were stored on BBC microcomputer disc via a video camera (Leslie, 1986). The responses of the spindle afferent to sinusoidal and ramp length changes with and without simultaneous stimulation of the gamma fibre were also recorded. The discharge of the passive, unstretched spindle was displayed as an interspike interval histogram generated with a CED1401 (Cambridge Electronic Design) intelligent interface connected to a Tandon 20 microcomputer. This system was also used for the generation of instantaneous frequency displays.

Twitch contractions of the soleus muscle were elicited by stimulation of the muscle nerve, at 0.1 s^{-1}. The resulting tension records were sampled and analysed by a microcomputer (3D09, Digital, Design & Development) running software under the FLEX operating system.

EU4093 was freshly prepared by dissolving 4 mg ml^{-1} in a carrier solution of sodium hydroxide, pH 10.3, made isotonic with 5% mannitol. This carrier solution has been shown to be without effect on extrafusal (Leslie & Part, 1981a) and intrafusal (Leslie & Part, 1981b) muscle contraction. In most experiments the drug was given in bolus injections via the external jugular vein to achieve concentrations of 5, 10, 15 or 20 mg kg^{-1}.

Results

The effect of EU4093 on extrafusal muscle twitch tension

A preliminary investigation of the effect of EU4093 on the isometric twitch contraction of the soleus muscle was made in three animals. In these experiments the tension transducer was rigidly attached to a micromanipulator whereas in the experiments designed to test the effect of the drug on intrafusal muscle the muscle had to be attached to the more compliant electromagnetic puller. The effects of the drug on twitch amplitude in the three experiments are shown in Figure 3. The aim of these preliminary experiments was to determine the dose of EU4093 to be used for the intrafusal work. It was decided on the basis of the results shown in Figure 3 to use a dose of 20 mg kg^{-1}, which gave a reduction of the

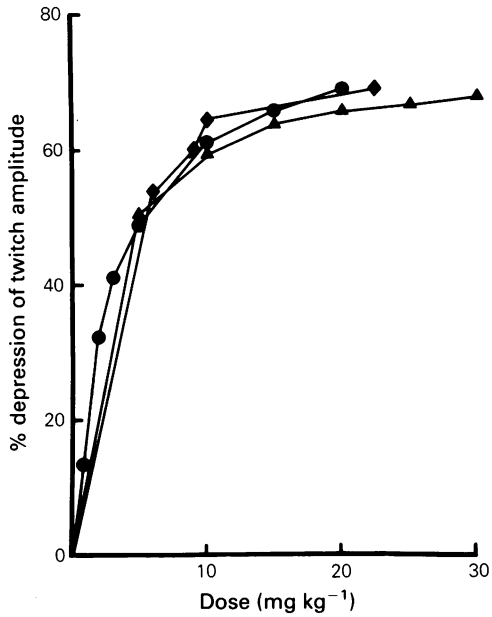

Figure 3 The effect of incrementally increased intravenous dose of EU4093 on the twitch amplitude of the soleus muscle stimulated supra-maximally via the muscle nerve at a frequency of 0.1 s^{-1}. The results of three individual experiments are shown.

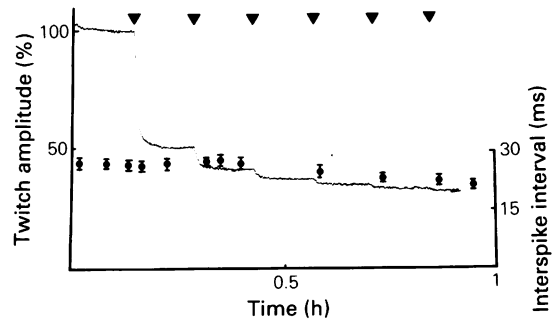

Figure 4 The effect of increased intravenous dose of EU4093 given at the arrows (▼) on the twitch amplitude of the soleus muscle and the interspike interval of a muscle spindle primary ending. The increment at each arrow was 5 mg kg^{-1} body weight, giving a final concentration of 30 mg kg^{-1}. The line shows the twitch amplitude with the muscle being stimulated once every 10 s via the muscle nerve. The interspike intervals are illustrated as the mean value of interval (●) and the range. It can be seen that there is no drug-related change in the interspike interval.

twitch amplitude to 31.9% of the control, a nearly maximal effect.

The time course of the action of EU4093 on extrafusal contraction is shown in the plots of Figure 4.

In ten experiments the effect of EU4093 on twitch amplitude was measured with the tension transducer mounted on the puller, the compliance of which precluded reliable results for the absolute values for the reduction of twitch amplitude. Nevertheless the relative dose-response curves were very similar to those obtained with the Devices transducer and confirmed the near maximum effect of a dose of 20 mg kg^{-1}.

The effect of EU4093 on the discharge of the passive spindle

If the contraction of intrafusal muscle is to be monitored from the discharge of the spindle sensory endings, it is essential to establish that the drug does not have a significant effect upon the discharge of the passive spindle. In three experiments interval histograms of muscle spindle primary discharge before and after the intravenous injection of each dose of EU4093 were recorded. The results of one of these experiments are shown in Figure 4. There is no drug-related change of interspike interval. In all the other spindle experiments additional data were obtained on the effect of EU4093 on the discharge of the passive spindle in the form of the control records of the discharge in response to ramp and sinusoidal stretch. None of these showed any evidence for an effect of the drug on the passive spindle.

The effect of EU4093 on the response of spindle afferents to gamma nerve stimulation

When first isolated a gamma nerve fibre was classified as static or dynamic (Crowe & Matthews, 1964) by means of the effect of stimulation of the gamma on the response to sinusoidal stretch. This test gives a clear demarcation between the two classes of gamma fibre to the rat soleus (Andrew *et al.*, 1978).

The response of the spindle afferent to stimulation of the classified gamma fibre at a range of frequencies with the muscle held at l_0 was recorded. The stimulation protocol was repeated after intravenous injection of EU4093. The effect of the drug on the discharge caused by gamma nerve stimulation varied considerably from preparation to preparation and so it is not appropriate to present mean results. Figure 5 shows graphs of the effect of EU4093 on the spindle discharge of a representative dynamic (Figure 5a) and static (Figure 5b) gamma fibre. It is apparent that the depression of intrafusal contraction as measured by spindle afferent discharge is dependent upon the frequency of stimulation. At low frequencies, at which there is just a slight increase in afferent frequency in the absence of the drug, EU4093 causes a total elimination of the

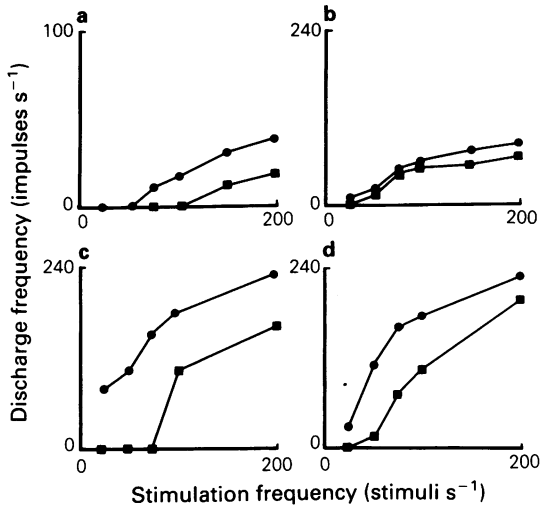

Figure 5 (a and b) Graphs showing the effect of EU4093 at a dose of 20 mg kg^{-1} body weight on the discharge frequency of muscle spindle primary afferents when a dynamic and static gamma fibre respectively are stimulated at frequencies between 25 and 200 stimuli s^{-1}; (c and d) show for comparison the results from similar previous experiments with dantrolene sodium (Leslie & Part, 1981b). Data obtained before (●) and after (■) injection of the drug.

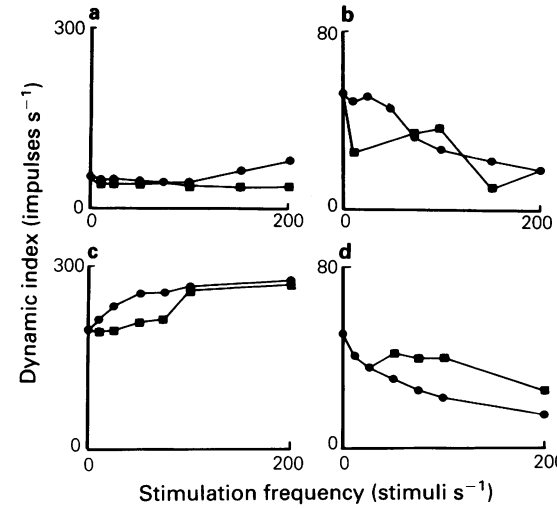

Figure 6 (a and b) Graphs showing the effect of EU4093 at a dose of 20 mg kg^{-1} body weight on the dynamic index of muscle spindle primary afferents when a dynamic and static gamma fibre respectively are stimulated at frequencies between 0 and 200 stimuli s^{-1}; (c and d) show for comparison the results from previous similar experiments with dantrolene sodium (Leslie & Part, 1981b). Data obtained before (●) and after (■) injection of the drug.

increase. At high frequencies of stimulation EU4093 still causes a similar reduction in frequency increase. In contrast the effect of dantrolene sodium was relatively less at high frequencies of stimulation than at intermediate frequencies; this is illustrated with previously published dantrolene sodium results (Leslie & Part, 1981b) plotted in the same manner (Figure 5c and d).

The effect of EU4093 on the dynamic index of the spindle response to ramp stretch was also investigated. The dynamic index is a measure of the dynamic sensitivity of a spindle ending (Crowe & Matthews, 1964). In general, stimulation of a dynamic gamma fibre will produce an increase in the dynamic sensitivity and hence dynamic index whereas stimulation of a static gamma fibre will cause a decrease in dynamic sensitivity.

As with the constant length experiments, the effect of EU4093 on dynamic sensitivity varied considerably from preparation to preparation. Figure 6 shows the effect of EU4093 on the changes of dynamic index brought about by the same static and dynamic gamma fibres as are shown in Figure 5. This figure illustrates the general finding that even at high frequencies (200 s^{-1}) of stimulation EU4093 causes a considerable reduction in the increase of dynamic index brought on by dynamic gamma fibre stimulation. The records of the response to sinusoidal stretch shown in Figure 7 also illustrate how EU4093 can have an appreciable effect on the increased modulation to stretch brought about by dynamic gamma nerve fibre stimulation at high stimulation frequencies. The dynamic gamma fibres investigated in this study happened not to bring about an appreciable increase in dynamic index until stimulated at quite high frequencies and therefore not too much significance should be read into the lack of change of dynamic index seen in Figure 6a.

Figure 6b shows the effect of EU4093 on the dynamic index with static gamma fibre stimulation. The striking feature of these results is the irregularity of the effect of increasing the stimulation frequency on the effectiveness of the drug. Figure 6c and d illustrate for comparison the typically much more regular relationship between stimulation frequency and effect of dantrolene sodium observed in previous experiments (Leslie & Part, 1981b).

Figure 7 illustrates that EU4093 altered the response of the spindle ending to stimulation of the static fibre at 200 s^{-1}; there is a slight decrease in the mean frequency of discharge with a considerable decrease in the reduction of modulation brought about by static gamma fibre stimulation. However with other gamma fibres the depth of the modulation

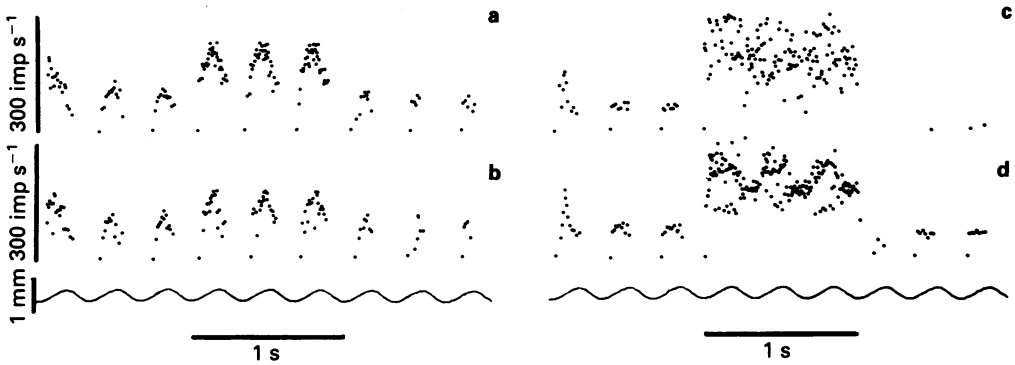

Figure 7 Instantaneous frequency displays of responses of muscle spindle primary endings to sinusoidal stretch before (a and c) and after (b and d) EU4093 at 20 mg kg^{-1}. Records (a) and (b) are with stimulation of a dynamic gamma fibre and records (c) and (d) with a static gamma fibre. Both fibres were stimulated at 200 stimuli s^{-1} for 1 s as shown by the bar.

to the stretch was about the same before and after the drug but the mean frequency of discharge was reduced.

Discussion

In a small number (3) of preliminary experiments we have shown that EU4093 depresses the twitch amplitude of the rat soleus muscle on average to 31.9% of the control at a dose of 20 mg kg^{-1} (body wt). This is a very similar result to that obtained with dantrolene sodium which depressed the soleus twitch to 31.3% of the control value at a dose of 5 mg kg^{-1} (Leslie & Part, 1981a). It is known that dantrolene sodium acts by means of inhibiting the release of calcium ions from the sarcoplasmic reticulum (van Winkle, 1976). Given the similarity of effect of EU4093 to that of dantrolene sodium on the twitch contraction of extrafusal muscle, it is probable that EU4093 acts in like fashion.

EU4093, like dantrolene sodium, has an effect on the contraction of intrafusal muscle. The effect of the drug is dependent upon the frequency of stimulation. At low frequencies the drug may abolish completely the acceleration of the spindle sensory ending. However, this does not necessarily mean that the drug has had the effect of totally preventing intrafusal muscle contraction as it may be that the contraction in the presence of the drug is below the threshold required to cause an acceleration of the afferent discharge. At high frequencies of stimulation the effect of EU4093 was rather different from that of dantrolene sodium in that EU4093 produced a pronounced depression of the acceleration of discharge frequency on stimulation of the gamma nerve fibre with the muscle held at constant length. This was observed in all the gamma fibres investigated, both static (7 fibres) and dynamic (5 fibres).

Dantrolene sodium has an effect on the dynamic index which is dependent upon the frequency of stimulation, with the greatest depression occurring at intermediate frequencies. The present results from EU4093 show that this drug had an unpredictable effect on the dynamic index of muscle spindle primaries as measured by the dynamic index in relation to stimulation frequency. For some gamma fibres EU4093 may cause a considerable reduction in the change of dynamic index brought about by stimulation of the gamma fibre, whereas for others EU4093 causes a minimal change of dynamic index.

The reduction of intrafusal muscle contraction caused by dantrolene sodium may be important in its overall muscle relaxant properties. A decrease in the response of spindle afferents to the gamma nerve activity will result in a decrease in excitatory input onto the motoneurones of the synergist muscles and hence a reduction in the extrafusal muscle contraction. Because of the form of the frequency-dependence of the effect of dantrolene sodium on the intrafusal contraction (Leslie & Part, 1981b), it is possible that the nervous system could overcome the muscle relaxant effect of dantrolene sodium on intrafusal muscle by increasing the frequency of discharge of the gamma nerve fibres. However, this is not a possibility with EU4093 because this drug still has an effect on intrafusal contraction at a stimulation frequency of 200 s^{-1}. In the decerebrate rat, dantrolene sodium does not have any effect upon the discharge of gamma nerve fibres to the de-afferented soleus muscle (Farquhar & Part, 1988). Hence in this preparation, at least, the nervous system is not compensating for the action of dantrolene sodium on intrafusal muscle and so the fact that EU4093 has a

greater effect than dantrolene sodium at high frequencies of discharge will not have any bearing on the relative muscle relaxant effect of the two drugs. If the gamma nerve discharge in the intact animal or human patient is as unresponsive to dantrolene sodium as it is in the decerebrate animal then in these also there should be no significant difference in the action of the two drugs in terms of their action on intrafusal muscle.

The effect of dantrolene sodium on extrafusal muscle is highly dependent upon the frequency of stimulation, with the action of the drug being minimal at high frequencies. At present there are no data on the effect of EU4093 on the tetanic contraction of extrafusal muscle in the anaesthetized rat. However in the anaesthetized and decerebrate rat the nervous system compensates for the effect of dantrolene sodium (Farquhar *et al.*, 1986; Farquhar & Part, 1988) only to a limited extent by increasing the frequency of discharge of the alpha motoneurones; more important are recruitment of additional motor units and modification of the patterns of discharge. It is possible therefore that dantrolene sodium and EU4093 would have a similar muscle relaxant action in the intact animal even if the effect of EU4093 on extrafusal muscle had a similar frequency dependency as that on intrafusal muscle.

In conclusion, it seems probable that EU4093 has a similar muscle relaxant action in the intact animal to dantrolene sodium despite the difference in the relation between the frequency of stimulation and the effect upon intrafusal muscle.

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