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DEPRESSION OF MUSCLE SPINDLE ACTIVITY— A NEW TYPE OF PHARMACOLOGICAL ACTION?

BY

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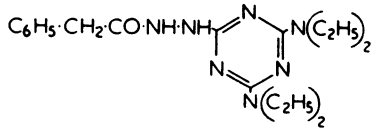
The effect of 2,4-di(diethylamino)-6-(2-phenylacetylhydrazino)-1,3,5-triazine (Ciba 28882-Ba) injected intravenously on the activity of de-efferented muscle spindles of the tibialis anterior and extensor digitorum longus muscles of anaesthetized cats, and on γ-motoneurones of decerebrate cats, was investigated. The drug depressed both the static activity of muscle spindles and the spontaneous activity of γ-motoneurones. In contrast, the response of muscle spindles to rapid stretch was not affected. The effect of 28882-Ba on the activity of the muscle spindles resembled that of progressive unloading or shortening of the muscle. 28882-Ba antagonized excitation of the muscle spindle by suxamethonium. The effect of 28882-Ba on the response of the γ-motoneurones to various driving stimuli was not predictable.

Muscle spindles together with their afferents and appurtenant γ-motoneurones, the γ-motoneurone-muscle spindle loops, constitute an important complex within the systems which control motor activity (Granit, 1955, 1959). Though the γ-motoneurone-muscle spindle loop plays an important physiological role, it has so far received remarkably little attention from the pharmacological point of view. In the course of investigations with triazine derivatives which inhibit strongly spinal reflex transmission (Bein, 1959) we made a systematic study of their effects on γ-motoneurones and muscle spindles and found among these derivatives some substances which, in view of their distinctive sites of action, might represent a new type of pharmacological effect. This report is concerned with the most active member of the triazine compounds investigated, namely Ciba 28882-Ba, and describes its effects on de-efferented muscle spindles and on γ-motoneurones. representing afferent and efferent branches of the γ-motoneurone-muscle spindle loops. Ciba 28882-Ba [2,4-di(diethylamino)-6-(2-phenylacetylhydrazino)-1,3,5-triazine] has the following structural formula:



Its mol. wt. is 371.50 and its m.p. is 132–134° C.

METHOD

All experiments were performed on adult cats of about 3 kg. The response of the muscle spindles was recorded from 34 receptors in 18 cats, and that of the γ-motoneurones from 10 units in 6 cats.

For the tests on de-efferented muscle spindles, cats were anaesthetized initially with ether and then with an allobarbitone-urethane mixture (Dial, Ciba) after cannulation of the trachea and V. jugularis externa. The dose of the mixture injected was usually 50 mg/kg intravenously, but in a few instances 40 or 60 mg/kg was administered. The muscle spindles investigated were those in the tibialis anterior and extensor digitorum longus muscles, of which the distal third was mobilized. The hind limb was denervated, except for the branches leading to the muscles in question, and was fixed rigidly in three places. A stimulating electrode was applied to the N. ischiadicus. After laminectomy and splitting of the dura mater, the spinal cord was covered with medicinal liquid paraffin at body temperature. Motor innervation of the muscles under study was eliminated by cutting the ventral roots of L_5 to S_3. Afferent fibres from muscle spindle endings were found in the dorsal roots of L_6 and L_7 and identified according to the criteria of Matthews (1933). Static and dynamic sensitivity of these endings was tested by stretching the muscle with a myograph (Fehr, 1962) and elongation was recorded photoelectrically.

The γ-fibres were studied in decerebrate cats. After cannulation of the trachea, the carotid arteries were temporarily clamped and the cats decerebrated under ether anaesthesia by midbrain transection (Sherrington, 1898). To differentiate the small diameter γ-fibres from the large diameter α-fibres, the relation between spike height and fibre diameter valid for fine strands was used (Hunt, 1951). From the severed ventral roots of L_7 and S_1 we selected filaments which, in addition to sustained rhythmical discharges of relatively low amplitude, also showed impulses of comparatively high amplitude either spontaneously or in response to pulling the Achilles tendon. By splitting these filaments further, we then isolated individual γ-fibres, the spontaneous activity of which fluctuated around a constant value. The reflex activity of the γ-motoneurones was tested by mechanical stimulation of the skin, by pinching the pinna (Granit, Job & Kaada, 1953), and by raising and lowering the head and tail. No attempt was made to determine the destination of the fusimotor fibres. With the exception of the ipsilateral ventral roots of L_7 and S_1 which were severed for recording, all peripheral nerves of the limb and all dorsal and ventral roots were intact.

For all tests on de-efferented muscle spindles and γ-motoneurones, the preparations were given artificial respiration. Pharmacological experiments on the γ-motoneurones were not begun until at least 4 hr had elapsed following discontinuation of the ether anaesthesia.

All injections were given into the external jugular vein or into a femoral vein. As it was poorly soluble in water 28882-Ba was dissolved in polyethylene glycol (average mol. wt. 400) and injected intravenously as 0.01 to 1% solutions. Control tests showed that polyethylene glycol alone exerted no effect in the experiments on de-efferented muscle spindles (in doses of up to 1 ml./kg intravenously) or on γ-motoneurones (in doses of up to 0.1 ml./kg). In the experiments with 28882-Ba, injections of 0.03 ml. to 0.1 ml./kg polyethylene glycol were employed and 0.1 ml./kg was never exceeded.

RESULTS

Effect on the discharge frequency of de-efferented muscle spindles

When the muscle was subjected to steady tension, 28882-Ba produced a prolonged reduction in the discharge frequency of de-efferented muscle spindles (Fig. 1). No initial increase in frequency was ever observed. The maximum effect after intravenous injection of 0.1 mg/kg of 28882-Ba was attained in 3.5 min. The time for 50% recovery averaged 67 min.

The effect of 28882-Ba was determined on two groups of endings with different functional characteristics but subjected to the same constant tension of 40 g. The first group represented endings of low threshold for steady discharges and low sensitivity to stretch, the second group those of high threshold and high sensitivity (not fully apparent in Fig. 2, since the threshold of most of the fibres was above 0 g).

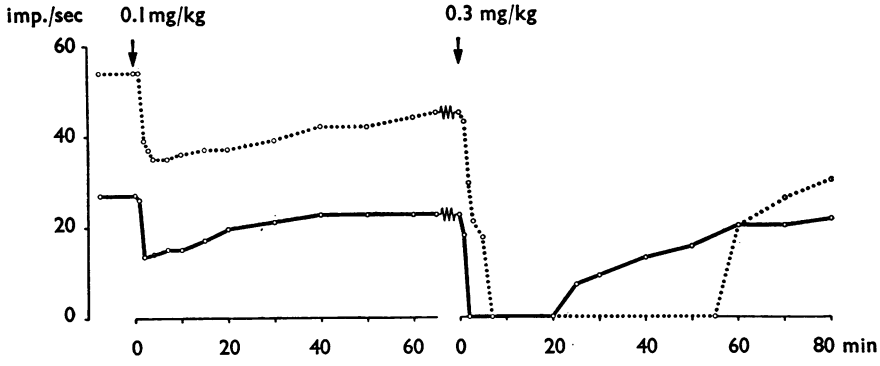


Fig. 1. Cat, allobarbitone-urethane anaesthesia, artificial respiration. Effect of 28882-Ba, in doses of 0.1 and 0.3 mg/kg intravenously, on the discharge frequency of two muscle spindle endings from a de-efferented tibialis anterior muscle under a steady tension of 30 g. Ordinate: Discharge frequency in impulses/sec. Abscissa: Time in min.

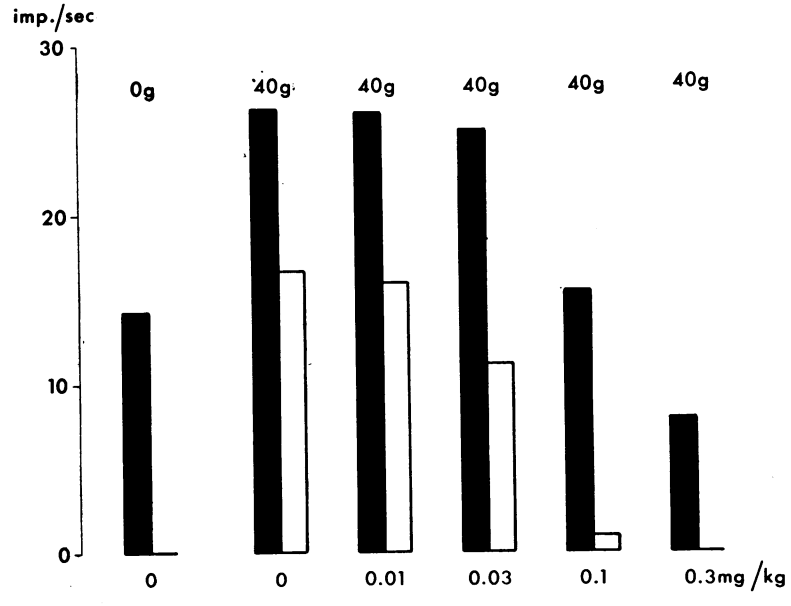


Fig. 2. Cats, allobarbitone-urethane anaesthesia, artificial respiration. Effect of 28882-Ba intravenously on the average discharge frequency from 10 muscle spindle endings with a low threshold and low sensitivity to stretch (sustained discharges at zero tension) ■; and from 10 endings with a high threshold and high sensitivity to stretch (silent at zero tension) □. Spindles were from the de-efferented tibialis anterior and extensor digitorum longus. Muscles were subjected to a steady tension of 40 g. Results were compiled from 11 experiments. Ordinate: Average discharge frequency in impulses/sec. Abscissa: (above) Load on muscles in g; (below: doses of 28882-Ba in mg/kg.

The conduction velocity of the afferent fibres was not determined ; their correlation to the fibre groups IA and II of Hunt (1954) thus remains uncertain. The average discharge frequency at a tension of 40 g was diminished by 0.1 mg/kg of 28882-Ba in the first group of low threshold by 11 impulses/sec or by 42% of the original value, whereas in the second group of high threshold it was diminished by 16 impulses/sec or by 95% (Fig. 2). The average frequency at a tension of 40 g after a dose of 0.1 mg/kg in both groups approximated to the frequencies recorded at zero tension before administration of the preparation. This indicates that 28882-Ba has essentially the same effect on both groups of endings, an effect resembling that of progressive unloading or shortening of the muscle, which would reduce the discharge frequency of the different endings according to their individual stretch frequency pattern.

Since the duration of action of 28882-Ba was prolonged, its influence on the stretch frequency pattern of the muscle spindles was studied by stretching the muscle at 1-min intervals from 0 to 2.5, 5 and 8, or 10 mm, respectively. On each occasion the muscle was stretched for 0.5 min and then allowed to return to relax for the next 0.5 min. The cycle was continued without interruption throughout the experiment. Stretches were performed by means of weights and stopped at the desired elongations by bars. High velocities of stretching were always employed. The arrangement permitted no shortening of the muscle.

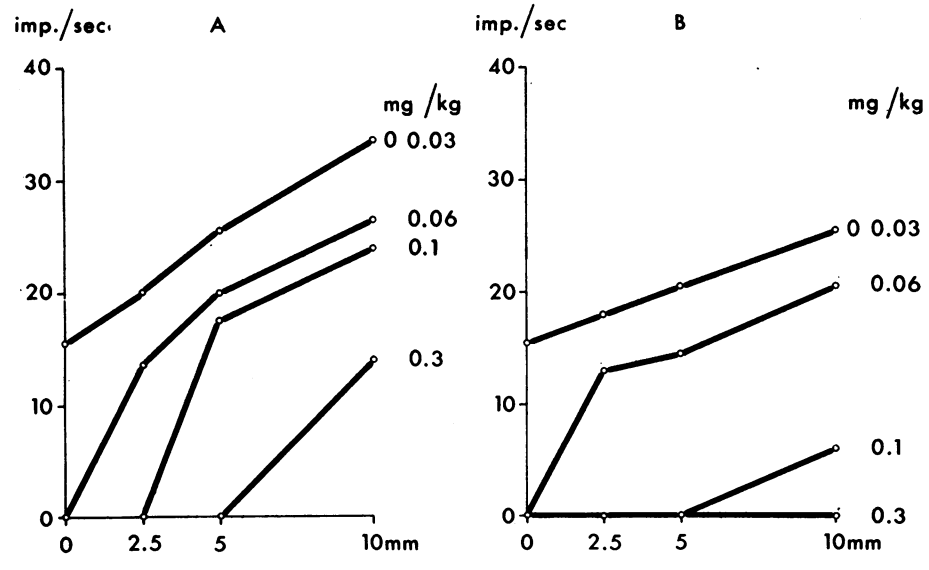


Fig. 3. Cat, allobarbitone-urethane anaesthesia, artificial respiration. Stretch frequency response pattern from a muscle spindle ending from the de-efferented tibialis anterior muscle at the time of maximum depression (2 to 5 min after injection) by various intravenous doses of 28882-Ba. All doses expressed in mg/kg. Abscissae: Extension of muscle measured in mm. 0 mm represents the muscle at rest. Ordinates: Discharge frequency in impulses/sec, in A 1 sec after phasic stretch and in B 30 sec after phasic stretch. The spontaneous discharge rate (30 sec after release from extension from 2.5 to 0 mm) was identical in A and B. 28882-Ba in a dose of 0.03 mg/kg intravenously had no effect. A dose of 0.3 mg/kg intravenously completely suppressed impulse discharge measured by procedure B.

Six endings in 3 preparations of this kind behaved similarly. A dose-dependent reduction in the static discharge rate occurred while at the same time the latter remained dependent on the degree of tension applied. Thus, not only the size of the dose but also the degree to which the muscle was stretched determined the discharge rate (Fig. 3). No irregularity of reduced static discharge was observed. After full recovery from depression of the static response, the threshold for steady discharge was in range of physiological threshold usually encountered. We did not make a systematic study of muscles stretched beyond 10 mm. In a few tests, 28882-Ba seemed to have a comparatively weaker inhibitory effect when very high tension was applied.

Compound 28882-Ba had a greater influence on the discharge frequency measured 30 sec after phasic stretch (Fig. 3B) than on the frequency as determined 1 sec after stretch (Fig. 3A). Little or no effect was exerted on the high discharge rate occurring during phasic stretch (Fig. 4). The stretch velocity was constant. It was particularly interesting that, with the aid of 28882-Ba, the dynamic phase could be separated *in vivo* from the static phase of afferent impulse discharge.

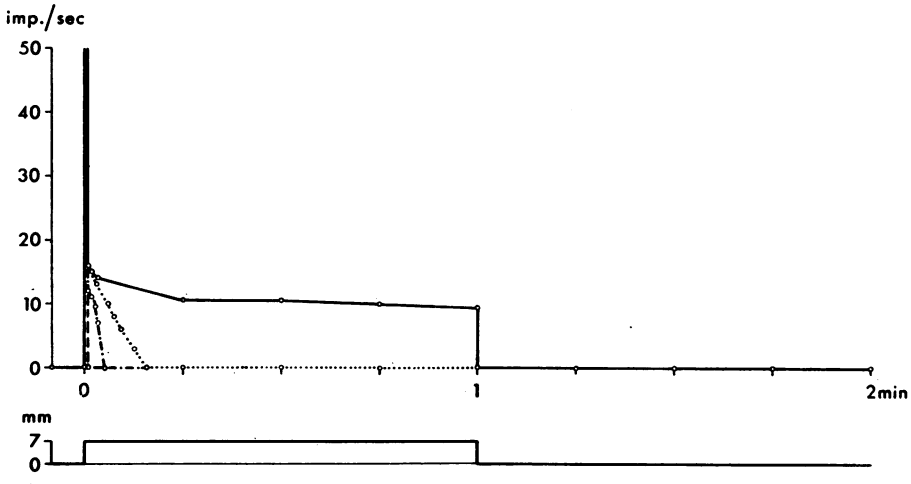


Fig. 4. Cat, allobarbitone-urethane anaesthesia, artificial respiration. Effect of 28882-Ba (0.1 mg/kg intravenously) on the discharge frequency of a muscle spindle ending from the de-efferented tibialis anterior muscle during phasic stretch and under steady tension. The muscle was stretched every 2 min from 0 to 7 mm by means of a 40 g weight for 1 min and was unloaded again for 1 min. —— Control response before 28882-Ba, and responses – – – – 4 min, –·–·– 60 min, and · · · · 100 min after injection of 28882-Ba in a dose of 0.1 mg/kg intravenously. Ordinate: Discharge frequency in impulses/sec and extension of muscle in mm. 0 mm represents the muscle at rest. The average discharge frequency during phasic stretch fluctuated around 250 impulses/sec both before and after 28882-Ba. Abscissa: Time in min.

Intravenous injection of suxamethonum activates muscle spindles (Granit, Skoglund & Thesleff, 1953). We investigated whether 28882-Ba could antagonize such pharmacologically-induced increases in frequency. Provided the injections were given at intervals of at least 20 min (Brinling & Smith, 1960), equal doses of suxamethonium elicited repeatable responses from the muscle spindles. Fig. 5 illustrates the antagonistic action of 28882-Ba on suxamethonium-induced excitation.

In a dose of 0.03 mg/kg i.v., which was not great enough to modify the impulse discharge in response to a constant stretch, 28882-Ba caused a decrease in the excitation following suxamethonium. In a few experiments, suppression of suxamethonium (0.1 mg/kg intravenously) induced activation by 28882-Ba (0.3 mg/kg) was overcome by increasing the dose of suxamethonium from 0.1 to 0.3 mg/kg.

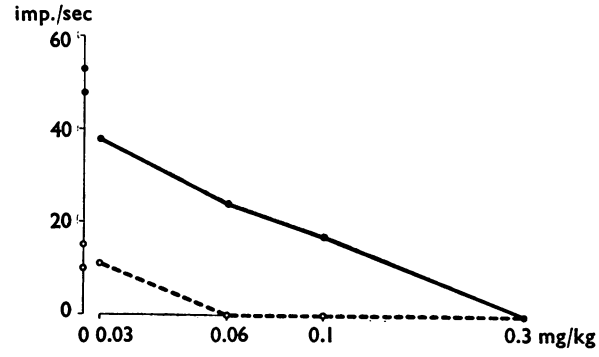


Fig. 5. Cat, allobarbitone-urethane anaesthesia, artificial respiration. Effect exerted by increasing intravenous doses of 28882-Ba on discharge frequency before excitation by suxamethonium (- - - -) and on the maximum discharge rate after suxamethonium (——) of a muscle spindle ending from the de-efferented extensor digitorum longus muscle under a steady stretch of 5 mm. Suxamethonium was injected every 20 min in a dose of 0.1 mg/kg intravenously, and on each occasion 28882-Ba was injected 5 min before the suxamethonium. Ordinate: Discharge frequency in impulses/sec. Each control value was a mean of two experiments. Abscissa: Doses of 28882-Ba in mg/kg intravenously on a logarithmic scale.

Effect on the discharge frequency of γ-motoneurones

In decerebrated cats, 28882-Ba decreased the spontaneous activity of γ-motoneurones in smaller doses than were required to decrease the activity of muscle spindles (Fig. 6). No initial increase in impulse discharge was observed in any of the 10 fibres whose activity was recorded in 6 preparations. The effect of 28882-Ba was reproducible, being dependent on the size but not on the sequence of the doses, even though the degree of rigidity resulting from decerebration was not identical in the preparations. Prior to administration of 28882-Ba, the fibres discharged 8 to 72 impulses/sec (mean, 29 impulses/sec). A dose of 0.01 mg/kg of 28882-Ba intravenously decreased the discharge frequency by a mean value of 27% compared with the initial value, and by a mean value of 92% when the dose was raised to 0.03 mg/kg. The maximum effect after 0.01 mg/kg was usually attained 3 min after the beginning of the injection while that after 0.03 mg/kg arose in 2 min. The time course of recovery was studied in some units silenced by 0.03 mg/kg. Impulses reappeared in 4 fibres after an average of 20 min from the beginning of the injection. Recovery of 6 fibres to 50% of the initial activity took an average of 24 min.

In contrast to its uniform effect on the spontaneous discharge of γ-motoneurones, the effect of 28882-Ba on the excitability of γ-motoneurones in response to

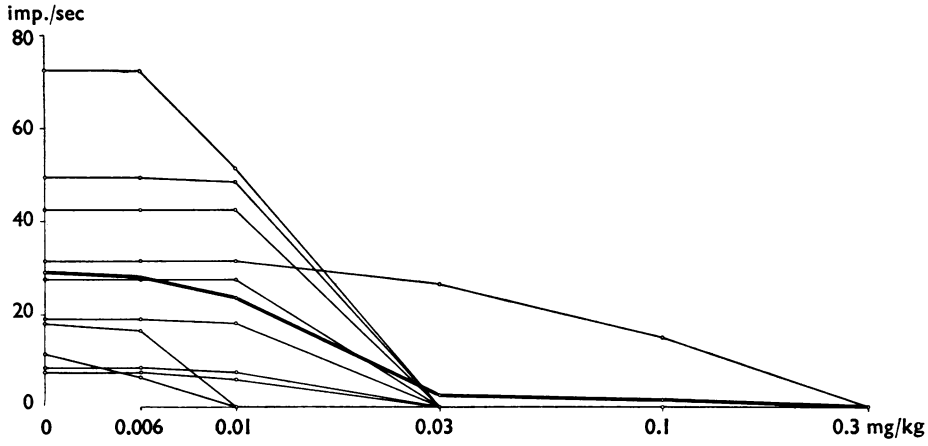


Fig. 6. Cats, decerebrated by midbrain transection, artificial respiration. Effect of 28882-Ba on the spontaneous discharge of 10 γ-motoneurones. Thin lines: single units; thick line: average value. Ordinate: Discharge frequency in impulses/sec. Abscissa: Doses of 28882-Ba in mg/kg intravenously (logarithmic scale).

mechanical stimulation of the skin and of the pinnae and to raising and lowering of the head and tail was less consistent. Activation of the various γ-motoneurones in response to the same form of stimulus varied during control periods before administration of 28882-Ba. The disappearance of spontaneous activity following intravenous 28882-Ba was usually accompanied by a diminution in reactivity as well. However, increases in reactivity were also observed—particularly, for example, after doses of up to and including 0.03 mg/kg, as well as at the beginning and in the recovery phase following higher doses, but only rarely throughout the entire duration of effect of higher doses. Occasionally reactivity to one form of stimulus ceased while that to another was enhanced. Thus, for instance, while one γ-motoneurone showed no reaction to stimuli applied to the pinna or tail after administration of 0.03 mg/kg of 28882-Ba intravenously, its reactivity to stimuli applied to the head increased by 150%. Following a dose of 0.1 mg/kg, its reactivity to the latter stimuli also disappeared. In our experiments we were unable to determine any relationship between the type of driving stimulus and the quality of the effect.

DISCUSSION

The receptor mechanism of the muscle spindle is controlled centrally. Muscle spindles respond to passive stretch. The rate of discharge of nerve impulses in response to a given muscle tension depends on the degree of contraction of the intrafusal muscle fibres. This contraction is initiated by the so-called γ motor fibres (Leksell, 1945 ; for a review, see Granit, 1955). In this elaborate control mechanism, part of the feed-back loop is peripheral and involves changes in the muscle spindles. The effect by 28882-Ba is twofold, firstly direct inhibition at the periphery and secondly by decrease of activity of the γ-motoneurones. There is no other substance of which we know that is capable of decreasing the activity of muscle spindles and

thus of specifically diminishing the afferent inflow of impulses into the spinal cord when administered systemically. Substances which have been described previously as exerting effects on the γ-motoneurone muscle spindle loop acted on its efferent branch by decreasing both the spontaneous activity and the reactivity of γ-motoneurones (for instance, chorpromazine and other phenothiazine compounds: Henatsch & Ingvar, 1956 ; Busch, Henatsch & Schulte, 1960 ; meprobamate: Busch, Henatsch & Schulte, 1960 ; general anaesthetic drugs: Andrew, 1961). In our own experiments with barbiturate drugs, chlorpromazine, chlordiazepoxide, and γ-aminobutyric acid, we detected no peripheral action on the muscle spindles (unpublished observation). Only after very large doses of mephenesin has depression of the static discharge of de-efferented muscle spindles been observed (Granit, in Granit & Holmgren, 1955). He did not, however, regard this as a specific effect and concluded that it was probably due to anoxia. When given in doses of 0.03 mg/kg intravenously and upwards, 28882-Ba produced transient decreases in blood pressure. A decrease in blood pressure does, however, not necessarily mean a diminution of local blood flow and consequent hypoxia. The sensitivity of muscle spindles to hypoxia is not known. The effect of anoxia induced by means of air embolism, however, set in about 3 min after circulatory arrest and was characterized by irregular increases and decreases of discharge frequency leading to final suppression some minutes later (unpublished observations). In contrast, the effect of 28882-Ba, started in the first min after injection, reached its maximum 3.5 min after 0.1 mg/kg, and the discharge remained regular. This strongly suggests that 28882-Ba has a specific action on muscle spindles. We have been unable to discover any analogous effects with substances other than triazine derivatives, even if they lower the blood pressure markedly (unpublished observations). Even intravenous injection of local anaesthetic drugs either failed to decrease muscle spindle activity altogether or failed to decrease it to the same marked degree (unpublished observations), as they depressed the activity of other stretch receptors such as the pulmonary stretch receptors (Bein & Bucher, 1957).

The complex structure of the muscle spindle poses the question as to which structural element in the spindle is affected by 28882-Ba. It is conceivable that diminution in impulse discharge in response to steady tension may be caused by a reduction in the sensitivity of the nervous receptors, by relaxation of the intrafusal muscle fibres, or alternatively by a combination of both these mechanisms. The situation is further complicated by the suggestion that there are various types of intrafusal muscle fibre (Diete-Spiff, 1961). Our experiments provide no conclusive evidence as to which type of mechanism is involved. From the finding that γ-motoneurone activity was decreased, it might be suggested that 28882-Ba influenced nervous substrates as such in a general manner. Not all peripheral receptors, however, are influenced by 28882-Ba ; for example, we were unable to influence the activity of pulmonary stretch receptors even with sublethal doses ; nor did 28882-Ba accelerate the adaptation of slowly adapting pulmonary stretch receptors (unpublished observation).

Suxamethonium is thought by Smith & Eldred (1961) to excite the muscle spindles by causing the intrafusal muscle fibres to contract, and Ottoson (1961) considers that

excitation by the drug of nervous elements in the muscle spindles of the frog is unlikely. But a diminution in the sensitivity of the receptors would still lead to a decrease in impulse discharge even if the contraction produced by suxamethonium were to remain unchanged. The suppression by 28882-Ba of the spontaneous discharge cannot be invoked to support the view that the compound exerts an action on the nervous receptors, for spontaneous discharge in cat spindles depends not on particular properties of the receptors but on muscle spindle tension when the muscle is at rest (Fehr, 1962). In this connexion it is of interest to note that, although the effect of 28882-Ba on impulse discharge from the spindle endings with different stretch frequency response patterns subjected to the same tension of 40 g appeared to be quantitatively the same as that produced by shortening the muscle, the compound appeared to have a less pronounced effect on discharge at extreme degrees of stretch. It is possible therefore that 28882-Ba might modify some mechanical factor the influence of which on the static mechanical behaviour of the intrafusal muscle fibres diminished as the spindles became further stretched. This suggestion is also supported by the observation that activity of a unit with a high spontaneous discharge rate of 21 impulses/sec (indicating a considerable tension of the spindle already in the muscle at rest (Fehr, 1962)) could not be completely silenced by the compound. Once the frequency had been reduced from 43 to 16 impulses/sec after 0.3 mg/kg intravenously, no further reduction occurred when an additional dose of 0.7 mg/kg intravenously was administered.

The implication of the finding that 28882-Ba had no effect on the dynamic phase of muscle spindle activity is not clear even assuming that the compound relaxed the intrafusal fibres or that it reduced the sensitivity of the nerve endings. Katz (1950) considers that the static and dynamic generator potential is dependent on various mechanisms, a conclusion which also seems to receive support from the investigations of Peruzzi & Corda (1961).

It has not been possible to determine the mechanism underlying the decrease of γ-motoneurone activity. Decrease in afferent inflow from muscle spindles as a possible explanation for the decrease in γ-motoneurone activity is excluded, since the latter was distinct in doses which failed to affect the muscle spindles directly. As 28882-Ba displayed neither sedative nor general anaesthetic properties in various animal species such as the mouse, rat, rabbit, cat, and dog (unpublished observations), its action cannot be based on general central depression.

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