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EFFECTS OF DIACETYL MONOXIME ON NEUROMUSCULAR TRANSMISSION

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The action of diacetyl monoxime on neuromuscular transmission has been studied in frogs, chickens, and cats, and in isolated rat phrenic nerve-diaphragm preparations. In frogs and chickens the oxime caused a flaccid paralysis; in chickens there was sometimes opisthotonos. In the indirectly stimulated rat diaphragm, diacetyl monoxime decreased the height of a single twitch, but a tetanus was well sustained. In cats, the twitch height of the indirectly excited gastrocnemius-soleus muscle was reduced by diacetyl monoxime more than was that of the tibialis anterior muscle, but in both muscles a tetanus was well maintained. Diacetyl monoxime reduced the response to direct stimulation of both the rat diaphragm and cat muscles. Diacetyl monoxime injected intra-arterially in the cat elicited a transient hypertension and a gasp. Diacetyl monoxime did not reverse the neuromuscular block caused by anticholinesterases either in isolated rat phrenic nerve-diaphragm preparations or in cats.

Amongst the oximes tested as antidotes against poisoning by organophosphorus insecticides, diacetyl monoxime has shown promising properties. Recently, Jager and Stagg (1958) have investigated the toxicity of diacetyl monoxime in man and Jager, Stagg, Green, and Jager (1958) its distribution in and excretion from the body. Wagley (1957) found that diacetyl monoxime decreased the end-plate potential in isolated amphibian muscle.

Since another active oxime, pyridine-2-aldoxime methiodide, is a neuromuscular blocking agent (Holmes and Robins, 1955; Fleisher, 1957), it was of interest to examine the effects of diacetyl monoxime on neuromuscular transmission.

METHODS

Frogs (*Rana ridibunda pallas*) of both sexes weighing 18 to 30 g. received injections into the dorsal lymph sac through the muscles of the hind limb, thus avoiding leakage of the injected solution. A volume of 0.04 ml./10 g. of body weight was used. Chickens weighing 90 to 140 g. of either sex, 3 weeks old, received injections (0.02 ml./10 g. of body weight) into the external jugular vein. All animals were observed for 24 hr. following injection.

Isolated phrenic nerve-diaphragm preparations (Bülbring, 1946), prepared from male white rats weighing 200 to 250 g., were placed in a 100 ml. bath containing Krebs solution at room temperature, bubbled with a mixture of 95% air and 5% CO_2.

Quantities of substances tested on this preparation are expressed in terms of final molar concentrations. Unless otherwise stated, indirect stimulation by rectangular pulses was of two kinds, either single supramaximal pulses of 0.5 msec. duration at a rate of 14/min. or tetanic stimuli at 350/min. applied for 5 sec. every 2 min. For direct muscle stimulation, single stimuli of 0.5 msec. duration were used.

Cats (of either sex) weighing 1.8 to 3.8 kg. were anaesthetized with ether followed by 80 mg./kg. of chloralose injected through a cannula in the external jugular vein. To record the contractions of the tibialis anterior and gastrocnemius-soleus muscles, the animals were prepared as described by Burn, Finney, and Goodwin (1950). The trachea was cannulated and spontaneous respiration was recorded from a rubber pneumograph tied around the chest and connected to a Marey tambour. The blood pressure was recorded from the right carotid artery with a mercury manometer; 20 mg. of heparin was injected to prevent clotting. About 15 to 20 min. before the injection of anticholinesterases, 0.5 mg. of atropine sulphate/kg. was administered intravenously and artificial ventilation was begun with a Palmer "Ideal" Pump. The vago-sympathetic trunks were cut in the neck. Electrical stimulation of the distal end of the cut sciatic nerve was carried out as for the phrenic nerve-diaphragm preparation; for direct stimulation of the muscles, the rod transfixing the femur was connected to the cathode, and a small stainless steel hook, which gripped the muscle tendons, was connected to the anode.

The oxime and anticholinesterases were injected intra-arterially through a metal cannula placed in the left

femoral artery, the iliac arteries having been ligated. The concentrations of solutions were so adjusted to give single injections of a maximum volume of 2 ml. for the oxime and 1 ml. for the anticholinesterases. The injections were made within 10 sec. of each other. For continuous injection of the oxime, a Braun slow infusion apparatus delivered 600 μg./0.5 ml./min. over 30 min.

Solutions of pure diacetyl monoxime and pure ethyl pyrophosphate (TEPP) were made with 0.9% NaCl and used immediately. Neostigmine methylsulphate and tubocurarine hydrochloride were obtained ready for use in sterile saline and diluted when necessary. All doses are expressed in terms of body weights.

Results

Diacetyl monoxime (100 mg./kg.) caused a flaccid paralysis and loss of the righting reflex within 40 min. in one of four frogs. This state lasted 15 min., after which the animal recovered its normal mobility. With 200 mg./kg. all of four animals were paralysed for from 20 to 105 min. Similar results were obtained on repetition of the experiments.

Two chickens injected with diacetyl monoxime (50 mg./kg.) showed transient paresis and weakness almost immediately but recovered within 1 min. Ten chickens injected with 100 mg./kg. developed a flaccid paralysis but recovered within 1 to 6 min. In three of these, opisthotonos, lasting 1 to 2 min., was also seen.

Twenty experiments were carried out on phrenic nerve-diaphragm preparations. When the muscle was stimulated with single shocks, concentrations of 1.25×10^{-3} M to 3×10^{-2} M of diacetyl monoxime caused a graded decrease in the height of contraction. With 4×10^{-2} M, there was a complete block. Direct stimulation of the muscle during the block elicited a contraction (Fig. 1) and a tetanus was also well sustained. Diacetyl monoxime also enhanced the blocking effect of tubocurarine (Fig. 2).

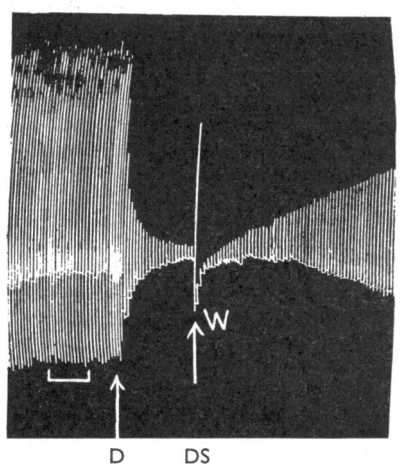

FIG. 1.—Effect of diacetyl monoxime on the isolated rat phrenic nerve-diaphragm preparation stimulated indirectly by single supramaximal stimuli, 0.5 msec., 14/min. At D, 1×10^{-2} M diacetyl monoxime was added; at DS, direct stimulation of the muscle. W, wash. Time, 1 min.

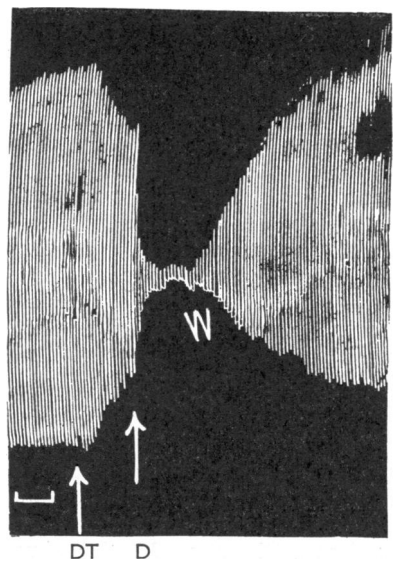

FIG. 2.—Isolated rat phrenic nerve-diaphragm preparation indirectly stimulated as in Fig. 1. Block caused by 1.27×10^{-6} M tubocurarine hydrochloride (DT) augmented by 1×10^{-2} M diacetyl monoxime (D). W, wash. Time, 1 min.

To examine the effect of diacetyl monoxime on the muscle fibre itself, experiments resembling those of Holmes and Robins (1955) were carried out. The preparations were maintained in a concentration of 1×10^{-2} M of diacetyl monoxime for 4 hr. and stimulated indirectly, with direct tetanic stimulation at intervals. With indirect stimulation there was an initial decrease in the height of contraction and no further change. The tetanus was always well sustained, and post-tetanic potentiation of the single twitch was seen. The response to direct stimulation was reduced after 2 to 3 hr. In another experiment in which the diaphragm, in 1×10^{-2} M diacetyl monoxime, was stimulated alternately directly and indirectly, the muscular response was also reduced, the indirect response being abolished before the direct.

In cats, single intra-arterial injections of 100 μg. and 5 mg./kg. of diacetyl monoxime had no effect

on the height of contraction of the tibialis anterior muscle, 5 mg./kg. produced only a slight effect on the response of the gastrocnemius-soleus muscles to single indirect stimuli. Doses of 30 mg./kg. of diacetyl monoxime invariably caused a decrease in the single twitch, the gastrocnemius-soleus muscle being affected more than the tibialis anterior muscle. Normal responses returned in 20 to 30 min. for the tibialis anterior muscle and 35 to 40 min. for the gastrocnemius-soleus muscle. With doses above 50 mg./kg., diacetyl monoxime completely blocked neuromuscular transmission in the gastrocnemius-soleus muscle and reduced activity in the tibialis anterior muscle to about 50% of normal. If, during the block, tetanic stimuli were applied the muscles gave a well-sustained response and a post-tetanic potentiation of the single twitch (Fig. 3). Direct stimulation of the muscles during the block caused a contraction.

After 30 mg./kg. of diacetyl monoxime a tetanus was always well sustained, although there was a marked reduction of the height of contraction of the gastrocnemius-soleus muscle and to a minor extent of the tibialis anterior muscle (Fig. 4). In some experiments, after the injection of 50 mg./kg. of diacetyl monoxime, the muscle responses to single and tetanic stimuli never completely recovered.

Effects on Blood Pressure and Respiration.—Single doses of 30 mg./kg. of diacetyl monoxime or more at times caused a transient rise in blood pressure of 30 to 70 mm. Hg (Fig. 3) followed by a brief hypotension (Fig. 4). After a deep gasp the respiration

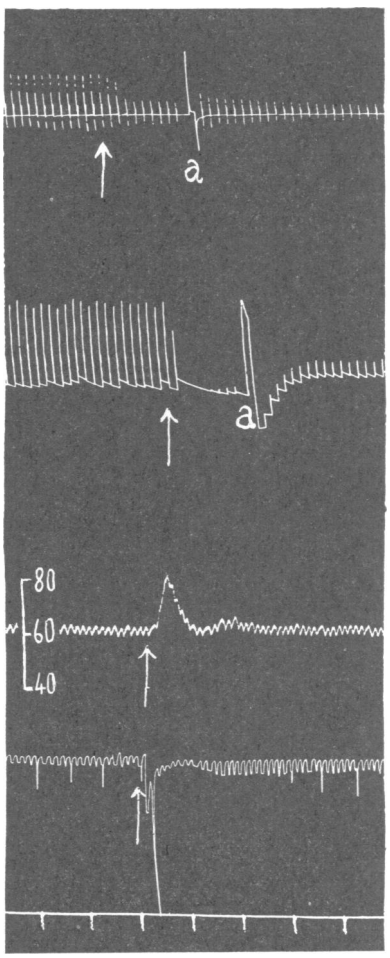

FIG. 3.—Cat, 2.5 kg., male. Records from above downwards: twitches of tibialis anterior muscle; twitches of gastrocnemius-soleus muscle; blood pressure (mm. Hg); respiration (inspiration downwards); time, 30 sec. Indirect stimulation (0.5 msec., 14/min.). At arrows, intra-arterial injection of diacetyl monoxime, 50 mg./kg.; at *a*, well-sustained contracture with tetanic stimulation for 5 sec. at 350/min.

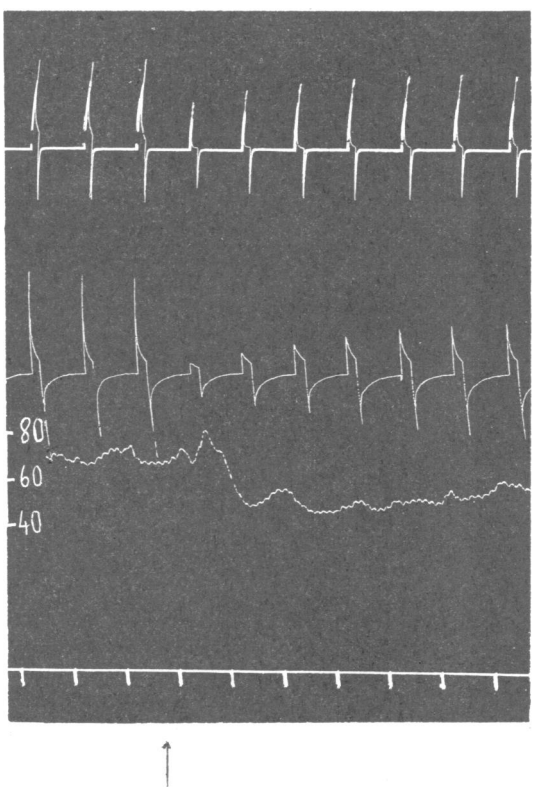

FIG. 4.—Cat, 1.8 kg., female. Records from above downwards: twitches of tibialis anterior muscle; of gastrocnemius-soleus muscle; blood pressure (mm. Hg); time, 30 sec. Indirect tetanic stimulation for 5 sec. every 30 sec. at 350/min. At arrow, intra-arterial injection of diacetyl monoxime, 30 mg./kg.

was usually rapid and shallow (Fig. 3) but sometimes continued normally. These responses, observed in 15 animals, have not been investigated further.

Effect of Diacetyl Monoxime on the Neuromuscular Block Caused by Anticholinesterases.—The action of diacetyl monoxime on the neuromuscular block caused by anticholinesterases was examined on isolated rat-diaphragm preparations and in cats.

In the rat diaphragm, two separate doses of ethyl pyrophosphate giving a total concentration of 1.7×10^{-5} M caused the usual enhancement followed by a decrease in the single twitches. In this latter state, diacetyl monoxime resulted in a further decrease (Fig. 5). When, instead of ethyl pyrophosphate, two doses totalling 6×10^{-6} M neostigmine methylsulphate were given, addition of diacetyl monoxime had the same result. During the period of enhancement of contraction, diacetyl monoxime antagonized ethyl pyrophosphate (Fig. 6). When two doses totalling 1.4×10^{-6} M ethyl pyrophosphate were added after 1×10^{-2} M diacetyl monoxime, the block caused by diacetyl monoxime was not reversed.

In another series of experiments, the diaphragm was stimulated tetanically every 2 min. When three doses giving a total concentration of 6×10^{-7} M ethyl pyrophosphate had been added at the start, after 7 and after 9 min., the muscle became unable to sustain the tetanus. Diacetyl monoxime (1×10^{-2} M) was then added and after 3.5 min. the preparation was washed. No important difference was found in the times required for the recovery of the tetanic response and that required without diacetyl monoxime, the times being 1 hr. 45 min. and 1 hr. 55 min.

With cats two types of experiment were carried out. In the first, 0.1 mg./kg. of ethyl pyrophosphate was injected, the sciatic nerve being given single stimuli. When the height of contraction of the tibialis anterior and gastrocnemius-soleus muscles had decreased, a single dose either of 100 μg. or of 5 mg./kg. of diacetyl monoxime was administered. In the second, tetanic stimulation was applied every 30 sec. and 0.25 mg./kg. of ethyl pyrophosphate was injected. When the muscles had lost their ability to sustain the tetanus, diacetyl monoxime was given as above or a slow infusion at 600 μg./0.5 ml./min. was given for 30 min. Control experiments in which ethyl pyrophosphate (0.25 mg./kg.) alone was injected showed that the tetanus was fully maintained again after 40 to 55 min. Sometimes, however, the recovery was not complete. In none of the above experiments did diacetyl monoxime influence the spontaneous recovery to normal height of single twitches or the ability to sustain a tetanus (Fig. 7).

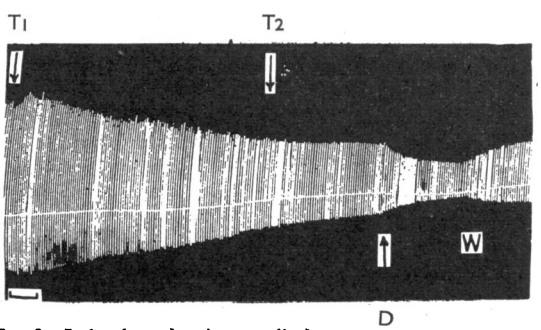

FIG. 5.—Isolated rat phrenic nerve-diaphragm preparation. Stimulation as in Fig. 1. At T_1 and T_2: 8.6×10^{-6} M and 1.7×10^{-5} M ethyl pyrophosphate respectively; at D, 1×10^{-2} M diacetyl monoxime. W, wash. Time, 1 min.

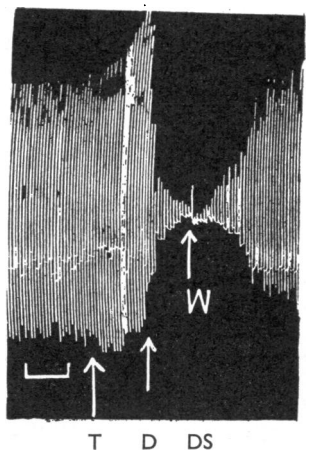

FIG. 6.—Rat phrenic nerve-diaphragm preparation. Stimulation as in Fig. 1. At T, 1.4×10^{-4} M ethyl pyrophosphate showing enhancement of single twitch. At D, 1×10^{-2} M diacetyl monoxime showing antagonism. At DS, contraction of the muscle after direct stimulation. W, wash.

DISCUSSION

The experiments showed that diacetyl monoxime, like pyridine-2-aldoxime methiodide, acted as a neuromuscular blocking agent in frogs, chickens, and cats and on the isolated rat phrenic nerve-diaphragm preparation. Grob and Johns (1958) have stated that diacetyl monoxime has no such effect in man. The type of block appeared to be of the competitive rather than the depolarizing type, but had some properties of the latter: for example, a flaccid paralysis was observed in all the chickens, but in some there was also opisthotonos. Two

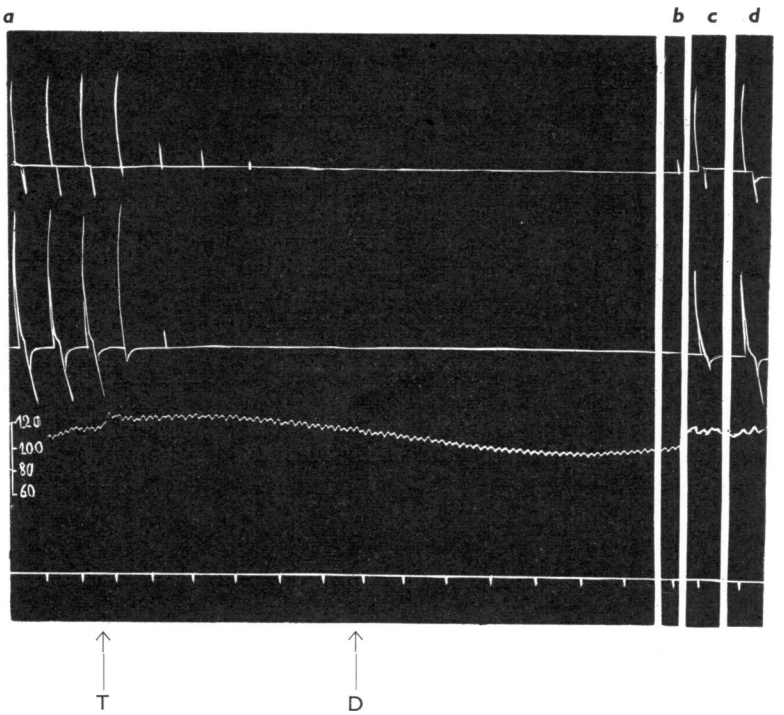

FIG. 7.—Cat, 2.0 kg., female, given atropine and artificially ventilated. Records from above downwards: tibialis anterior muscle; gastrocnemius-soleus muscle; blood pressure (mm. Hg); time, 30 sec. Indirect tetanic stimulation for 5 sec. every 30 sec. at 350/min. At T, ethyl pyrophosphate (0.25 mg./kg.) intra-arterially causing a gradual loss of ability to maintain a tetanus. At D, start of continuous intra-arterial infusion of diacetyl monoxime (600 μg./0.5 ml./min. for 30 min.). At *b*, *c*, and *d*, tracings obtained 10, 34, and 53 min. after commencement of diacetyl monoxime infusion. Note that diacetyl monoxime did not influence the spontaneous recovery of the ability to maintain tetanus.

effects of diacetyl monoxime upon the striated muscle of the same human subject have been reported by Jager and Stagg (1958), who found clonic movements of the head at the same time as flaccid paralysis of the limbs.

In cats, the gastrocnemius-soleus muscle was more affected than the tibialis anterior muscle, as with curare-like drugs, but a tetanus was always well sustained. In the isolated diaphragm preparation, diacetyl monoxime decreased the twitch both during the enhancement and subsequent block caused by anticholinesterases.

Diacetyl monoxime appears to act mainly at the end-plate region, as direct muscle stimulation always elicited a contraction both in the isolated preparation and the anaesthetized animal. Some effect on the muscle fibre itself is also indicated, however, because sometimes the initial height of contraction was not fully regained.

It is well known that anticholinesterases first cause an enhancement and later a decrease or block of twitch responses in striated muscle stimulated indirectly. The ability to sustain a tetanus is also suppressed. Monoisonitrosoacetone (Holmes and Robins, 1955) and pyridine-2-aldoxime methiodide (Holmes and Robins, 1955; Wills, Kunkel, Brown, and Groblewski, 1957), relieve the neuromuscular block caused by anticholinesterases. Grob and Johns (1958) have reported that diacetyl monoxime in minute quantities (50 to 100 μg.) promptly and strikingly reverses the local neuromuscular block produced by anticholinesterases in man. With these and even larger quantities, this effect of diacetyl monoxime in cats and rat phrenic nerve-diaphragm preparations was not observed here. Holmes and Robins (1955) have stated that the reversal of neuromuscular block is entirely due to a reactivation of inhibited cholinesterase in the muscle. Since diacetyl monoxime is a poor reactivator compared with pyridine-2-aldoxime methiodide and monoisonitrosoacetone, its failure to reverse the block, which is a peripheral phenomenon, is not surprising. Thus diacetyl monoxime appears to have a more marked central than peripheral action in protecting against anticholinesterase poisoning. The same authors have also found that some oximes given close-intra-arterially failed to reverse the neuromuscular block elicited by anticholinesterases, because the oximes were not " fixed " in the muscle. This does not seem to be true of diacetyl monoxime, since a relatively small single dose (5 mg./kg.) caused a slight decrease in the twitches, indicating that the substance was " fixed."

The ability of diacetyl monoxime to penetrate the blood-brain barrier has been demonstrated by Jager *et al.* (1958); this possibly explains why it acts as a synergist to pyridine-2-aldoxime methiodide (Edery and Schatzberg-Porath, 1958). With regard to tubocurarine, diacetyl monoxime appears to act differently from the oximes used by Holmes and Robins (1955), for they found that these oximes have no effect on the block caused by tubocurarine. In our experiments diacetyl monoxime and tubocurarine were synergic.

In cats the rise in blood pressure produced by a single injection of diacetyl monoxime, which was found by Jager and Stagg (1958) in man, was also observed here.

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