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THE REACTIONS OF RABBITS TO POISONING BY *p*-NITROPHENYLDIETHYLPHOSPHATE (E600)

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The compound *p*-nitrophenyldiethylphosphate (E600) is an active inhibitor of acetylcholine esterase, and under the conditions in which this reaction has been studied *in vitro* the inhibition of the cholinesterase of red blood cells is apparently irreversible (Aldridge, 1950). Hobbiger (1951) has shown that the inhibition of red cell cholinesterase by tetraethylpyrophosphate is slowly reversible if observations are continued for a long enough period. Comparable observations with E600 have not been made.

The experiments recorded in this paper suggest that some reversal of the inhibition of cholinesterase in the animal may take place very rapidly and that successive inhibition by repeated doses of E600 can also be reversed. But simultaneously other irreversible changes are also taking place.

METHODS

Male rabbits of mixed breeds weighing 1–2.8 kg. were used. A Palmer " Ideal " respiration pump was used to provide the artificial respiration.

In order to facilitate artificial respiration in the unanaesthetized animals tracheotomy was sometimes performed under ether and a glass cannula tied in place. The wound was sewn up and the animal allowed to recover fully (one hour) from the effects of the ether.

Observations were made on the respiratory movements, the carotid arterial blood pressure, and the response of the tibialis anticus and soleus muscles to indirect electrical stimulation of rabbits anaesthetized with urethane 1 g./kg. intravenously. Ether was given only during the preparation of the tendons of the leg muscles immediately after preliminary tracheotomy. Under urethane alone the sciatic nerve was exposed and tied, and silver-wire electrodes placed beneath it. The nerve was stimulated once every two seconds by a square wave impulse of supramaximal intensity and 0.4 m.sec. duration. A lever attached to the liver by a thread recorded movements of the diaphragm. At the end of the dissection a further 0.5 g./kg. of urethane was given ; no further anaesthetic was required. All drugs were given by the ear vein and all the animals were given repeated doses of atropine. Stock solutions of E600 in absolute alcohol were diluted either in saline or atropine immediately before use. Acetylcholine was administered as a freshly prepared solution in saline.

RESULTS

Reactions of Unanaesthetized Rabbits to E600

A single dose of 0.1 mg./kg. E600 given intravenously produced effects within 1–2 min. characteristic of poisoning by drugs which resemble acetylcholine. Atropine was given to the majority of animals in order to prevent salivation, bronchial secretion, bronchial spasm, and cardiac slowing. Widespread muscular fasciculations appeared and the animal collapsed with frequent kicking movements of the limbs. Respiration ceased and was soon followed by circulatory collapse as judged by the condition of the ear vessels. Death took place within 10–15 min. of injecting E600. But if artificial respiration was administered before the circulation collapsed, spontaneous breathing started within 2–5 min., and as it improved in amplitude and frequency the convulsive limb movements gradually disappeared. Within 25 min. of administering the E600 the animal could sit up, but showed an obvious general weakness and persistent widespread fasciculations.

As many as 10 successive doses of E600 have been given to a single rabbit over a period of $2\frac{1}{2}$ hours. Each was followed by a rapid collapse and generalized limb twitchings, but with the help of artificial respiration the animal was restored to consciousness.

The periods of unconsciousness did not appreciably increase with successive doses. Other changes did appear. The generalized fasciculations but not the convulsive limb twitchings were very much reduced after several doses of E600 and almost disappeared. The failure of respiration

after 2–3 doses of E600 took a new form. Instead of a sudden arrest with progressive return after artificial respiration, breathing gradually slowed and became more shallow. Natural breathing could be restored by a period of artificial respiration, but might fail again in the same way several times before recovery was complete. The need for longer and more frequent periods of artificial respiration in order to restore natural respiration increased with successive doses of E600.

Anaesthetized Rabbits

Within 1 min. of injecting 1 mg./kg. E600 muscular fasciculations became visible and soon spread to involve most of the musculature. The movements of the diaphragm became very irregular until they ceased altogether, except for some fasciculations. At this stage the costal muscles might begin to operate and a few heaving chest movements take place. These soon ceased, cyanosis rapidly became extreme, and the blood pressure began to rise. After a further 1–2 min. the blood pressure fell rapidly and the heart slowed and soon stopped. During this asphyxial period the muscular fasciculations became very intense and twitching of the limbs sometimes occurred. If artificial respiration were started as soon as the blood pressure began to rise, recovery was certain, but this was not always so if the start of artificial respiration was delayed until the fall in blood pressure had begun. Immediately on starting artificial respiration by forced ventilation there was a sharp fall in blood pressure, presumably the result of interfering with the venous return to the heart by raising the intrathoracic pressure, but the colour improved and within a few minutes normal respiratory movements started. At first they were small in extent, but soon increased, so that the earlier rhythm and excursions were restored. As soon as oxygenation was improved tremors and fasciculations diminished, but the latter persisted after the full restoration of natural breathing.

If a succession of doses of E600 was given at intervals of 30–60 min. the diaphragm became refractory and ceased to respond by fibrillations and failure. Of nine rabbits receiving repeated doses of E600 in this way, four became refractory after one dose, four after two doses, and one after three doses of E600. Successive doses of E600 then had no immediate effect at all upon the excursions of the diaphragm. However, over the ensuing 3–5 hours the respiration rate gradually became slower and the diaphragm excursions smaller. There was also a slow but continuous fall in blood pressure unaffected by further doses of atropine. This gradual failure of both the respiration and circulation finally became complete and the animals died. The course of the decline could be temporarily arrested by prolonged periods of artificial respiration, which temporarily improved the blood pressure and the rate and extent of the diaphragm excursions. Seven animals receiving four or more injections of E600, totalling from 4 to 13 mg./kg., survived for 170–310 min., but the length of survival was not proportionate to the amount of drug administered.

The response of the leg muscles to indirect stimulation varied somewhat from animal to animal. But in all preparations there was some diminution of the contraction after each dose of E600, and this response was seen even after the diaphragm had become refractory to the drug.

The other most obvious change was the disappearance during the later stages of the poisoning of all the fine muscular fasciculations.

If it is assumed that E600 kills the animals because of the ability to inhibit cholinesterase, it is necessary to postulate that during the short periods of artificial respiration some at least of this process is reversed. Evidence that some cholinesterase might be released from inhibition was provided by the response of the rabbit to acetylcholine. A dose of 100 μg. acetylcholine given intravenously to rabbits that had received atropine had no effect upon the respiratory movements or response of the leg muscles to indirect stimulation.

When this same dose of acetylcholine was given to a rabbit revived by artificial respiration from a "lethal dose" of E600, the response of the stimulated leg muscles and natural respiratory movements were abolished or reduced for varying periods. The severity of the reaction diminished as the interval between the time of injecting the E600 and the acetylcholine was increased. If given 20 min. after E600, 100 μg. acetylcholine would arrest respiration and abolish the response of the leg muscles. But 100 min. later the same dose of acetylcholine had no effect on respiration and only reduced the response of the leg muscle by 10–15%.

When the animal was given a series of injections of E600, the response to 100 μg. acetylcholine given at the same interval after each dose of E600 did not change. Even though the natural respiration became unaffected by the later doses of E600 it remained equally sensitive to acetylcholine given after the E600.

One difference only was noted in the response of the rabbits to acetylcholine given after successive doses of E600. After the first or second dose of E600, acetylcholine produced a transient

shower of muscular fasciculations in the whole musculature, but this did not happen when the acetylcholine was given after later doses of E600. By this time spontaneous muscular fasciculations had also ceased.

Discussion

De Candole, Douglas, and Spencer (1950) studied rabbits poisoned by tetraethylpyrophosphate, which has an action very similar to that of E600. They found that at the time when the animal died the diaphragm no longer responded to indirect stimulation by way of the phrenic nerve. Death appeared to be due to a failure of nerve conduction at the periphery. This is apparently a very temporary failure after E600, though it may suffice to kill the animal in the absence of artificial respiration. If the diaphragm is inspected during the phase of acute poisoning it is seen to be the seat of irregular fasciculations, which are also widespread throughout the voluntary musculature.

Soon after the start of artificial respiration, with improved oxygenation of the peripheral blood the general fasciculations are much reduced, though they persist to some extent. After a few minutes of artificial respiration spontaneous respiratory movements start. Gradually the spontaneous fasciculations in the body musculature die down and at this stage injections of E600 no longer affect the excursions of the diaphragm.

Lundholm (1949) studied the effects of DFP on the rabbit and stated that there were two types of respiratory failure, one due to a failure of nerve-muscle conduction at the periphery and a second later stage which he attributed to central effects. Repeated doses of E600 in both anaesthetized and unanaesthetized rabbits result in a gradual slowing of respiration and failure of the circulation, which are unaffected by injections of atropine and are presumably due to the action of E600 on the central nervous system.

It is necessary to consider the role played by the inhibition of cholinesterase in the production of these signs of poisoning. Judged by the response of the anaesthetized rabbit to injections of acetylcholine, an inhibition of cholinesterase takes place after each of a succession of doses of E600. This inhibition is to some extent reversed in the course of the next two hours. But in two hours the reversal is never enough to restore the animal's normal degree of insensitivity to acetylcholine. Successive " lethal " doses of E600 do not make the animals more sensitive to acetylcholine, which suggests that the inhibition after the first dose is maximal. Reversal of this sensitivity to acetylcholine takes place with equal readiness after each of a series of injections of E600. Although the movements of the diaphragm are unaffected by the later injections of E600, they are still arrested by acetylcholine, which, if given within 10 min. of a dose of E600, may produce respiratory arrest of sufficient duration to require artificial respiration to prevent death.

The generalized muscular fasciculations gradually disappear after successive doses of E600. If the appearance and subsequent disappearance of these fasciculations are related to the degree of inhibition of cholinesterase at the myoneural junction, it is clear that a reversal of this effect is certainly not taking place in the intervals between the successive doses of E600. If the interval between doses is extended to 1–2 hours the fasciculations do return, and if no further doses of E600 are given will continue uninterruptedly for a further hour or two. During the early stages of poisoning the occurrence of these fasciculations apparently interferes to such an extent with the response of the diaphragm to natural and artificial impulses that complete failure of respiration occurs. But if the animal is tided over this critical period by artificial respiration, the diaphragm recovers sufficiently to respond to regular natural stimuli. This condition is not brought on again by successive doses of E600. As the death of the animal can be attributed to asphyxia, it is not unreasonable to suppose that asphyxia itself may make the condition at the diaphragm so much worse for the conduction of natural impulses from the phrenic nerve. This supposition is supported by the observation that throughout the voluntary musculature the fasciculations are intensified by the asphyxia and are immediately relieved when artificial respiration redresses the condition. Paton and Zaimis (1951) noted a similar effect on cats injected with decamethonium. The " curarizing " effect of this drug was enhanced during periods of anoxia due to respiratory failure. Apparently under conditions of asphyxia myoneural conduction is less efficient than when the blood is fully oxygenated. In the rabbit poisoned with E600, after 1–3 doses the diaphragm apparently overcomes this handicap to the conduction of natural impulses, and with the disappearance of the fasciculations further inhibition of cholinesterase by successive doses of E600 does not upset conduction.

In the unanaesthetized rabbit successive doses of E600 produce periods of unconsciousness accompanied by convulsive limb movements. If the inhibition of cholinesterase is responsible for the

production of these effects, this inhibition, too, must be readily reversed during the period of artificial respiration after each dose of E600. The duration of the period of unconsciousness is not significantly increased after each of a number of successive doses of E600.

Thus in anaesthetized and unanaesthetized animals sensitivity to acetylcholine and the production of unconsciousness go through a similar reversible cycle after a number of successive doses of E600, and these effects might be attributable to periodic complete inhibition of the cholinesterase at the periphery and in the central nervous system followed by a partial reversal of this inhibition.

In both types of animals slow progressive changes also take place, associated with a gradual slowing of respiration and failure of the circulation. It seems probable that these must be the result of some secondary changes brought about by a persistence of a considerable degree of cholinesterase inhibition in the tissues.

SUMMARY

1. Rabbits, anaesthetized or unanaesthetized, die from acute respiratory failure within 10–20 min. of receiving a lethal dose of the powerful anticholinesterase *p*-nitrophenyldiethylphosphate (E600). The failure is apparently peripheral and can be overcome by a short period of artificial respiration.

2. Revival by artificial respiration may be effected after a series of such injections in the unanaesthetized rabbit.

3. After 1–3 such injections the anaesthetized rabbit no longer responds to further doses of E600 and respiration does not stop.

4. Gradual failure of the circulation and respiration develops after a series of injections of E600.

5. Sensitivity to acetylcholine increases after each dose of E600, and diminishes but does not return to normal within a 2-hour period.

6. Muscular fasciculations are widespread after the early injections of E600, but disappear and do not reappear after further doses of E600.

7. While part of the effect of E600 on cholinesterase can be reversed in the living animal, the development and disappearance of the muscular fasciculations are not part of a reversible cycle.

8. It is suggested that some of these changes may be a secondary reaction to the inhibition of cholinesterase.

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