Brit. J. Pharmacol. (1962), **18**, 19–28.

EFFECTS OF ORGANOPHOSPHOROUS COMPOUNDS, OXIMES AND ATROPINE INJECTED INTO THE THIRD VENTRICLE OF UNANAESTHETIZED DOGS

BY

H. EDERY*

From the Israel Institute for Biological Research, Ness-Ziona, Israel

(*Received June* 14, 1961)

The effects of four organophosphorous compounds, three oximes and atropine sulphate, injected through an indwelling cannula into the third ventricle of unanaesthetized dogs were examined. The effects of 200 μg of dyflos were involuntary micturition, defaecation, akinesia of hind limbs and pronounced disturbances of awareness; those of 100 μg of ethyl pyrophosphate were tremor, restlessness and signs of fear; 500 μg to 5 mg of dyflos and 250 μg to 500 μg of ethyl pyrophosphate caused vomiting, salivation, twitches of facial muscles and recurrent epileptiform seizures. The injection of 40 to 80 mg of dimefox and of 50 mg of schradan elicited involuntary micturition, vomiting, salivation and defaecation. These effects occur probably after these substances have passed into the blood stream and have been converted in the liver to potent anticholinesterases. This view is supported by the finding of reduced blood cholinesterase activity. At a dose level of 12.5 mg, 1,1'-trimethylenebis(4-hydroxyiminomethylpyridinium bromide) produced strong convulsions. At this dose level pralidoxime iodide and diacetyl monoxime produced no observable effects. Atropine sulphate in a dose of 1 mg caused disturbances in consciousness and behaviour followed by convulsions. Intraventricular atropine and to a minor extent intraventricular oximes were able to antagonize the effects of intraventricular ethyl pyrophosphate. Pralidoxime iodide exerted a strong antagonistic effect also on intravenous injection.

Intoxication with organophosphorous compounds is known to cause psychological disturbances and other central effects in man (Wilson, 1954; Holmes & Gaon, 1956; Hayes, Dixon, Batchelor & Upholt, 1957; Grob & Harvey, 1958; Hiraki & Namba, quoted by Wills, 1959), and in cats pronounced behavioural changes have been obtained after injection of dyflos into the lateral cerebral ventricle (Feldberg & Sherwood, 1954b; Yokota, 1959).

In recent years, oximes have been tested on animals as possible antidotes against organophosphorous poisoning. Two compounds, pralidoxime iodide and diacetyl monoxime, have been used successfully in man. Their efficacy was so far investigated only after systemic administration of large amounts of organophosphates. As these produced both peripheral and central actions it was difficult to evaluate their

* Present temporary address: National Institute for Medical Research, Mill Hill, London, N.W.7.

effectiveness as antidotes on the central effects elicited. It seemed possible to overcome this difficulty by injecting small doses of organophosphates into the cerebral ventricles of unanaesthetized animals, even if the central disturbances elicited by this route of administration need not be identical with those produced when the substances reach the brain via the blood stream. The present experiments were therefore undertaken in order to investigate the effects of intraventricular injections of organophosphorous compounds of oximes and of atropine in unanaesthetized dogs; and, further, to find out if oximes and atropine were able to antagonize the effects of intraventricular ethyl pyrophosphate.

METHODS

In 33 adult mongrel dogs of both sexes an indwelling cannula was implanted under pentobarbitone anaesthesia into the third cerebral ventricle and fixed in the left parietal bone. The method was that described by Haley & Dickinson (1956). Bleeding from the skin and muscular layers was kept to a minimum by infiltrating adrenaline dissolved in saline solution at several points around the operation site. Wax was used to stop bone bleeding. Seven to ten days elapsed between operation and the first intraventricular injection. At that time the external wound was partially healed and the animals showed no abnormalities in behaviour.

The organophosphorous compounds used were dyflos (di-isopropyl phosphorofluoridate), ethyl pyrophosphate (TEPP), dimefox (*NNN'N'*-tetramethylphosphorodiamidic fluoride), schradan (bis-*NNN'N'*-tetramethylphosphorodiamidic anhydride; octamethyl pyrophosphoramide, OMPA). The oximes were pralidoxime iodide (pyridine-2-aldoxime methiodide; 2-hydroxyiminomethyl-*N*-methylpyridinium iodide; PAM), diacetyl monoxime and 1,1'-trimethylenebis(4-hydroxyiminomethylpyridinium bromide). Atropine was given as the sulphate. All the substances were freshly dissolved in saline solution. The volume of each intraventricular injection was 0.25 ml. At least 3 dogs received each dose of the substances tested. In some experiments, two injections were carried out, in one dog at intervals of 5 min. After injection, the animals were left for 6 to 8 hr unrestrained in the room or on a chain long enough to permit free movement; afterwards they were kept in separate cages. Each trial on the same animal was separated by an interval of 3 to 10 days.

In some experiments the cholinesterase was determined in blood samples obtained from the saphenous vein. The samples were stored in heparinized tubes until the blood cholinesterase activity was determined by the method described by Fleisher, Pope & Spear (1955). Results are expressed in mM of acetylcholine hydrolysed by 0.1 ml. of blood during 20 min incubation at 25° C.

RESULTS

Saline

Intraventricular injections of 0.25 ml. saline solution did not produce any changes in behaviour of the dogs, except that there was occasionally sniffing at objects placed in the room and urination 1 to 2 min after injection.

Dyflos

The injection of 200 μg dyflos produced the following effects: about 2 min after injection the dogs urinated in a fashion unusual for male animals, that is, without raising their leg. This was termed involuntary micturition. After 4 to 6 min the animals became restless and ran continuously in an apparently purposeless manner, avoiding anyone who tried to stop them. Passage of liquid stools occurred; 5 to 7

min later a gradual akinesia of the posterior limbs developed ; the animals sat down and were unable to stand. Although the leg muscles lost their tone, the dogs resisted attempts to put them in abnormal positions. The animals remained sitting in a stuporous condition, watching aimlessly for periods of 10 to 15 min. They did not react to noise or caresses and made no movement of withdrawal when a finger was put close to their eyes. Their state of consciousness appeared to be disturbed. The corneal reflex was unaltered. About 30 min after injection the animals recovered, but there was occasional scratching of the face.

The effects cannot be attributed to inhibition of blood cholinesterase since this enzyme was found to be inhibited to a slight degree only, if at all. As seen from Table 1 (dogs 1 and 2), 16 hr after the injection the blood cholinesterase was inhibited less than 20% which is within the normal limits of daily fluctuations. Since inhibition of blood cholinesterase with dyflos is irreversible there could not have been greater inhibition at the earlier stages when the behavioural changes were observed.

The injection of 500 μg to 5 mg of dyflos produced vomiting within 2 to 3 min. Then the dogs scratched their faces and there was involuntary micturition ; within the next few minutes there was continuous salivation, tachypnoea and twitching of the facial muscles mainly around the eyelids and mouth ; the animals gave the impression of being frightened and then fell in generalized tonic-clonic convulsions. Salivation increased ; there was micturition and loss of stools. This condition, which lasted 2 to 4 min, closely resembled an epileptiform attack ; afterwards the dogs stood up, became restless and irritable. Sometimes they showed aggressive behaviour, attempting to bite people approaching. The animals recovered within 0.5 hr. After the injections of 5 mg of dyflos several epileptiform seizures occurred at intervals of 8 to 15 min. About 15 hr after the injection, the dogs were comatose and had continuous twitching of the facial muscles ; they died a few hours later. In one dog the injection of 5 mg of dyflos elicited somewhat different symptoms. There was extreme hyperexcitability 7 min after the injection. The dog barked continuously in a strange fashion. At times it bit furiously at inanimate objects such as the trough and the walls. A pronounced mydriasis developed. The dog did not recognize familiar persons but tried to bite them when approached. It drank water repeatedly. The pupils returned to their normal size 15 min after injection, but the rage-like state lasted for about 1 hr. No convulsions developed and the animal recovered within 2 hr.

Ethyl pyrophosphate

A few minutes after the injection of 100 μg of ethyl pyrophosphate the dogs became tremulous and restless and seemed to want to run out of the room. The animals were alert and aware of their surroundings throughout. Some dogs vomited, passed liquid stools, salivated and repeatedly scratched their face, back and abdomen. About 20 to 40 min after the injection they returned to their normal condition.

In a dose of 250 μg and 500 μg ethyl pyrophosphate elicited practically the same effects as those produced by 500 μg to 5 mg of dyflos: salivation, facial twitching, clonic-tonic convulsions and later a condition of irritability. A few dogs remained

stuporous during the intervals between seizures and did not develop the state of irritability. In some animals penile erection occurred.

Since the intraventricular injection of 200 μg of this organophosphate which produces long-lasting inhibition of cholinesterase did not reduce significantly the blood cholinesterase determined 24 hr after the injection (see dogs 3 and 4, Table 1). the effect is most likely the result of inhibition of the brain cholinesterase in structures near the ventricular surfaces.

After the injection of 500 μg of ethyl pyrophosphate death occurred within 18 hr, and during this period there were four or five seizures. Pentobarbitone sodium (25 mg/kg) given via the saphenous vein abolished the convulsive activity and the animals recovered within 24 hr. One animal, however, died 18 hr after receiving 250 μg of ethyl pyrophosphate although the pentobarbitone sodium had abolished the convulsions.

Dimefox

Dimefox in a dose of 500 μg or 2 mg did not cause any noticeable effects in behaviour. In three animals 40 mg of dimefox was injected; in two dogs no abnormalities were seen; the third urinated and shook its head several times, scratched its face vigorously and rubbed its body against the floor; later there was salivation. The effects disappeared about 30 min after the injection.

In three dogs the injection of 80 mg of dimefox elicited after about 20 min involuntary micturition and vomiting. About 30 min later, the animals passed fluid stools, salivated, became restless and repeatedly scratched their face and body. Two hours after injection salivation was still present and there was rectal and vesical tenesmus. General awareness seemed unaffected, but the animals were apathetic and had a tendency to lie down. Twenty-four hr after injection one dog died, the other two recovered. In one of these three dogs which had received 80 mg dimefox the cholinesterase was determined in blood samples taken 0.5 hr and 2 hr after the injection. In both samples a 35% inhibition was found. This finding shows that some of the compound must have entered the general circulation within the first 30 min. However, from the finding shown in Table 1 (dogs 5 and 6) that 24 hr after an intraventricular injection of half of the amount of dimefox the inhibition of blood cholinesterase was 56 and 71% respectively, it is evident that the full inhibiting effect on blood cholinesterase had not occurred during the first 2 hr. Since dimefox has no anticholinesterase activity *per se* but acquires this property after being metabolized in the liver, the behavioural effects observed cannot be attributed to inhibition of brain cholinesterase on penetration of the compound into the ventricular walls. The effects must therefore either be due to a direct action of the dimefox on the central nervous system independent of brain cholinesterase inhibition or brought about after the compound had entered the general circulation and been converted into a potent inhibitor of cholinesterase in the liver.

Schradan

The injection of 50 mg of schradan, another organophosphate which becomes an inhibitor of cholinesterase after being metabolized in the liver, caused micturition

and defaecation 5 to 7 min after the injection, and salivation lasting 30 min. The animals then returned to normal. The blood cholinesterase was found to be inhibited by almost 50%, 24 hr after the injection (dogs 7 and 8, Table 1).

TABLE 1

BLOOD CHOLINESTERASE ACTIVITY AFTER INTRAVENTRICULAR INJECTION OF ORGANOPHOSPHATES

Cholinesterase activity of blood immediately before (I) and 16 to 24 hr after (II) the injection of organophosphates. Cholinesterase activity expressed in mM of acetylcholine hydrolysed by 0.1 ml. blood incubated at 25° C for 20 min

Dog no.	Organophosphate	Dose in mg	Cholinesterase activity (I)	Cholinesterase activity (II)	% inhibition
1	Dyflos	0·5	2·2	1·8	18
2	Dyflos	0·5	1·4	1·2	14
3	Ethyl pyrophosphate	0·2	1·8	1·6	10
4	Ethyl pyrophosphate	0·2	2·0	1·8	10
5	Dimefox	40·0	1·6	0·7	56
6	Dimefox	40·0	2·8	0·8	71
7	Schradan	50·0	1·8	1·0	44
8	Schradan	50·0	2·3	1·1	52

Oximes

Intraventricular administration of pralidoxime iodide (12.5 mg), diacetyl monoxime (12.5 mg) or of 1,1′-trimethylenebis(4-hydroxyiminomethylpyridinium bromide) (6.5 mg) produced no obvious changes in the behaviour of the dogs. After 12.5 mg of the dioxime there occurred involuntary micturition, vesical tenesmus and continuous salivation within 30 min of injection, followed by dyspnoea as well as fasciculation and twitching of limb and facial muscles. After about 1 to 2 hr, generalized tonic-clonic convulsions developed, lasting 4 to 5 min.; during the convulsions there was probably loss of awareness of the surroundings. Pentobarbitone sodium administered intravenously stopped the convulsions; the animals recovered within 24 hr, except one animal which died during this time.

Atropine sulphate

The effects of intraventricular injection of 500 μg atropine sulphate did not differ from those of saline solution, but after 1 mg of atropine most animals began barking continuously in an unusual fashion within 5 to 7 min, whined at intervals and looked about apparently without cause. The animals did not recognize familiar people and became unresponsive to noise. This condition lasted from 0.5 to 1 hr; then the dogs appeared to become afraid; they pulled at their chains in an attempt to escape. There was tachypnoea and salivation. Four to five epileptiform seizures of 2 to 3 min duration occurred at intervals of 5 to 7 min. During the intervals, the animals seemed to be conscious, breathed rapidly, salivated and sometimes whined. Later the dogs again began barking in an unusual manner and were running about; at times they suddenly stopped and looked as if they were facing some danger and then made sudden movements of escape. The impression was gained that the dogs were having hallucinations.

Within 4 hr of injection the animals returned to their normal condition.

Intraventricular atropine and oximes on the effects of intraventricular ethyl pyrophosphate

The effects of ethyl pyrophosphate (250 μg) were abolished or reduced if they were followed 5 min later by an intraventricular injection of atropine or of an oxime. Atropine was the most, and the oximes, 1,1′-trimethylenebis(4-hydroxyiminomethylpyridinium bromide) and diacetyl monoxime, the least, effective antagonists.

When atropine sulphate (500 μg) was injected into 3 dogs after the ethyl pyrophosphate, two showed no symptoms at all and the other only slight salivation 8 min after the injection.

When pralidoxime iodide (12.5 mg) was injected into 3 dogs after the ethyl pyrophosphate the convulsions were prevented and awareness did not seem to be impaired, but the following symptoms occurred: salivation, restlessness, passage of fluid stools. The effects were over within 1 hr.

When 1,1′-trimethylenebis(4-hydroxyiminomethylpyridinium bromide) (6.25 mg) and diacetyl monoxime (12.5 mg) were injected each into three dogs after the ethyl pyrophosphate, one seizure occurred in each dog. Recovery was complete within 2 hr.

Intravenous oximes on the effects of intraventricular ethyl pyrophosphate administration

The oximes were injected in a 5% solution through the left saphenous vein 1 to 2 min after the ethyl pyrophosphate. Diacetyl monoxime (25 mg/kg) did not modify the ethyl pyrophosphate symptoms; it did not prevent the convulsions or the other symptoms and did not delay the lethal outcome; the animals were dying about 1 hr after the injection. 1,1′-Trimethylenebis(4-hydroxyiminomethylpyridinium bromide) (20 mg/kg) had some protective effect. Out of four injected dogs only two developed seizures, and each had only two seizures apparently with loss of awareness. In addition there was slight salivation, tremor and hyperpnoea; recovery was complete within 3 hr. Pralidoxime iodide (50 mg/kg) was the most effective antagonist. No convulsions or signs of impaired awareness were observed, and the sole effects were slight salivation, tremor and hyperventilation which occurred a few minutes after the injection and disappeared within 0.5 hr.

DISCUSSION

A number of substances are able to penetrate into the cerebral tissue from the ventricular cavities. After intraventricular injection of organophosphate anticholinesterases the cholinesterase activity of brain tissue surrounding the ventricular cavities was found to be greatly reduced in dogs (Burgen & Chipman, 1952) as well as in rabbit (Koelle & Steiner, 1956). Penetration into the ependyma and subependymal tissue has also been observed for such different substances as a fluorescent dye (Rodriguez, 1955), radioactive protein (Bowsher, 1957), histamine (Draskoci, Feldberg, Fleishhauer, & Haranath, 1960) and bromophenol blue (Feldberg & Fleischhauer, 1960). It is reasonable to assume that the central effects elicited by intraventricular injections of anticholinesterases, oximes and atropine result from an action on structures lining the cerebral ventricles.

The effects of intraventricular injections of small doses of dyflos in dogs resembled those seen in cats (Feldberg & Sherwood, 1954b ; Yokota, 1959), but they were comparatively milder. Itching and catatonia-like state, severe in cats, were less pronounced in dogs. This may be due to species differences or to differences in the site of absorption of the substance. In the present experiments the injections were made into the third ventricle, whereas the results on cats were obtained with injection into the lateral ventricle. With large doses, however, there was no difference in the symptomatology seen in dogs and cats.

Both dyflos and ethyl pyrophosphate produced convulsions when injected by the intraventricular route, but ethyl pyrophosphate was found to be more potent. This is readily understood, as this organophosphate is a more potent anticholinesterase. The convulsions probably result from accumulation of undestroyed acetylcholine since intraventricular acetylcholine also produces convulsions (Feldberg & Sherwood, 1954a ; Traczyck, 1959). Although in the present experiments the anticholinesterases were injected into the third ventricle the possibility cannot be excluded that they diffused partly into the lateral ventricles and produced the convulsive activity by an action on structures lining these cavities since the convulsive activity of intraventricular tubocurarine would be explained in this way (Feldberg & Fleischhauer, 1960). Other actions of the anticholinesterase, however, may be on diencephalic structures reached from the third ventricle. In this connexion it is interesting to note that in man lesions in the wall of the third ventricle are associated with loss of consciousness (for references see Brain, 1958) and that the penile erection seen in dogs following intraventricular ethyl pyrophosphate may be due to an action on structures in the septum pellucidum, since its electrical stimulation elicits this effect in monkeys (MacLean, Ploog & Robinson, 1960).

The inability of dimefox and schradan to elicit convulsions is not surprising. These organophosphates are weak inhibitors of cholinesterase *per se* ; to be converted to potent anticholinesterases they must first pass through the liver (du Bois, Doull & Coon, 1950 ; Okinaka, Doull, Coon & du Bois, 1954). Since brain is unable to perform this conversion (Fenwick, Barron & Watson, 1957) lack of central effects after intraventricular injection of even large doses of these compounds is readily understood, and those effects which were seen after intraventricular injection of these compounds are probably not the result of penetration of these substances into the brain tissue from the ventricular walls but occur after they have passed into the blood stream and have been converted into potent anticholinesterases.

The fall in blood cholinesterase activity when these symptoms appear supports this view. Moreover, on intravenous injection in dogs the dose of schradan used in the present experiments is near the LD50 (du Bois *et al.*, 1950), and the dose of dimefox would produce strong parasympathetic stimulation (Okinaka *et al.*, 1954).

Pralidoxime iodide and diacetyl monoxime injected intraventricularly were without effects in dogs. In man, these drugs cause dizziness, convulsions and loss of consciousness on intravenous administration (Wills, 1959). This difference may be accounted for by the fact that different sites of the brain are reached by the two routes of administration.

1,1′-Trimethylenebis(4-hydroxyiminomethylpyridinium bromide) was more toxic than pralidoxime iodide and diacetyl monoxime when injected intraventricularly.

It produced seizures and death in doses in which the other oximes produced no noticeable effects. In large doses given intravenously to dogs this compound produces convulsions (Bay, 1959).

The symptoms observed after injection of atropine in dogs resemble those in cats (Feldberg & Sherwood, 1954a), but in dogs the response is closer to that in man. In the present experiments the intraventricular injection of 0.5 mg produced no observable effects in dogs, and this dose is apparently also insufficient when injected intraventricularly in man (Cushing, 1931). On the other hand, the convulsions produced in dogs by large doses recall the convulsive activity of atropine poisoning in man. In cats intraventricular atropine apparently does not produce convulsions (Feldberg & Sherwood, 1954a). Finally, some of the disturbances in the behaviour of the dog suggested hallucinations which are a common feature in man following an overdose of atropine.

Both atropine and some of the oximes had the ability to counteract some of the central effects of intraventricular ethyl pyrophosphate. The effect of atropine is readily explained as an antagonist of the central action of acetylcholine. Atropine exerts this effect also on intraventricular injection in man (Henderson & Wilson, 1936) and cats (Feldberg & Sherwood, 1954a). For the antagonism of the oximes reactivation of the brain cholinesterase may be the main or sole mechanism responsible. For instance, Tong & Way (1960) found that in mice intraventricular pralidoxime iodide alleviated the effects of ethyl pyrophosphate, and Longo, Nachmansohn & Bovet (1960) found that pralidoxime iodide delayed the appearance of the pattern of " grand mal " elicited by sarin in the electroencephalogram of curarized rabbits. The original idea that pralidoxime iodide, being a quaternary compound, does not cross the blood-brain barrier no longer holds true, as Jager, Stagg, Green & Jager (1958) detected pralidoxime in rabbit brain after intravenous injection of the oxime and Rosenberg (1960) obtained evidence of reactivation of brain cholinesterase inhibited by paraoxon in certain circumscribed areas of the rabbit brain. The possibility that other mechanisms apart from reactivation of cholinesterase may contribute to the antagonistic effects of these compounds is envisaged by Lindgren & Sundwall (1960).

In the present experiments pralidoxime iodide counteracted the convulsions produced by ethyl pyrophosphate more effectively than 1,1′-trimethylenebis(4-hydroxyiminomethylpyridinium bromide). This might be due to greater ability of pralidoxime to penetrate the brain. Nevertheless, in different experimental conditions, 1,1′-trimethylenebis(4-hydroxyiminomethylpyridinium bromide) has been reported to be more effective than pralidoxime iodide as antidote for organophosphate poisoning (Fleisher & Michel, 1959 ; Hobbiger & Sadler, 1959). The almost complete inability of diacetyl monoxime to antagonize ethyl pyrophosphate is in agreement with previous findings that this oxime does not antagonize the toxic action of the anticholinesterase in mice, nor the neuromuscular block caused by the organophosphate in cats (Edery & Schatzberg-Porath, 1958 ; Edery, 1959).

My thanks are due to Mrs G. Schatzberg-Porath and Mr I. Zahavi, who assisted me in some of the experiments, and to Drs Z. Pelchowicz and S. Cohen for the supply of the organophosphates and oximes used in this investigation.

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