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# A CENTRAL VASODEPRESSOR EFFECT OF DYFLOS

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1 Cats were anaesthetized with pentobarbitone sodium and atropinized peripherally by intravenous injection of atropine methyl nitrate; the effect was examined of topical bilateral application of dyflos to the ventral surface of the medulla oblongata at a region lateral to the pyramids and caudal to the trapezoid bodies. Dyflos was applied by means of perspex rings; the volume of fluid placed in each ring was 10 $\mu$l.

2 The topical application of dyflos (1-20 mg/ml) produced a fall in arterial blood pressure without changes in heart rate and, in experiments without artificial ventilation, tachypnoea with dissociation of thoracic and abdominal respiration.

3 Atropine methyl nitrate (50 mg/ml) applied topically in the same way as dyflos, prevented or abolished its vasodepressor effect.

4 The two reactivators of acetylcholinesterase, obidoxime (100-200 mg/ml) and pralidoxime mesylate (100-200 mg/ml), applied topically in the same way as dyflos, abolished its vasodepressor effect. The reactivator compound 30 (100 mg/ml), also a pyridinium aldoxime, did not have this effect.

5 Obidoxime and pralidoxime mesylate also reversed the vasodepression produced by carbachol applied to the ventral surface of the brain stem but not the vasodepression produced by glycine similarly applied.

6 The problem is discussed as to whether the reversal of the dyflos and carbachol-induced vasodepression by obidoxime and pralidoxime is due to acetylcholinesterase reactivation by dephosphorylation and decarbamylation respectively, to a central atropine-like action of these compounds or to a combination of both.

## Introduction

Recently it was shown that large doses of the anticholinesterase dyflos (di*iso*propyl phosphorofluoridate) could produce, in anaesthetized rabbits, a fall in arterial blood pressure which was not prevented by atropine. This atropine-resistant vasodepression was found to be not peripheral in origin and thought to be due to an action 'within the neuronal vasomotor pathway'. No indication was given as to whether the action was on the central nervous system or on sympathetic ganglia (Preston & Heath, 1972). It seemed possible that dyflos acted on a region at the ventral surface of the brain stem which contains 'chemosensitive zones', since another inhibitor of acetylcholinesterase, physostigmine, produced vasodepression when applied to this region in anaesthetized cats (Guertzenstein, 1973).

To test this possibility the method developed for topical application of drugs by means of perspex rings onto this region (Feldberg & Guertzenstein, 1972; Guertzenstein, 1973) was used in the present experiments. Dyflos produced a fall in arterial blood pressure when applied to this region. The effect was resistant to the peripheral component of the action of atropine, but was prevented or abolished by atropine methyl nitrate when, like dyflos, it was applied topically. The effects of topical application of oximes which are reactivators of acetylcholinesterase were also examined on the depressor effect of dyflos.

## Methods

Male cats weighing between 3.7 and 4.9 kg were anaesthetized by i.p. injection of pentobarbitone sodium (30 mg/kg), supplemented whenever required later in the experiment by an i.v. injection of 5 mg/kg. For intravenous injections the left

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femoral vein, and for recording arterial blood pressure, the left femoral artery, were cannulated. The blood pressure was recorded on a Smith's Servoscribe potentiometric recorder with a transducer connected through a Cambridge preamplifier (Type 72342). For counting heart rate the blood pressure was recorded at a fast speed. The trachea was cannulated and if not otherwise stated, the cats were artificially ventilated throughout the experiment with a Palmer respiratory pump, and atropinized peripherally with an intravenous injection of 2 mg/kg of atropine methyl nitrate, which does not readily pass the blood-brain barrier.

The method of applying drugs bilaterally by means of perspex rings to the ventral surface of the brain stem was that developed by Feldberg & Guertzenstein (1972) and recently described in detail with a diagram of the perspex rings and their holder by Guertzenstein (1973). The perspex rings were placed so that each covered a nearly round area of about 5 mm diameter, lateral to the pyramids and just caudal to the trapezoid bodies. The drugs were placed in each ring in a volume of 10 $\mu$l and removed after 5 min by repeated washing of the area inside the rings with artificial CSF. The correct position of the perspex rings was checked at the end of the experiments by placing a 0.8% solution of bromophenol blue in the rings, killing the cat by an overdose of intravenous pentobarbitone sodium a few minutes later, rapidly washing out the dye from the rings before removing them from the brain stem, and observing the stained areas with the naked eye. Figure 1 shows the staining obtained in an experiment in which the rings had been placed correctly.

*Drugs*

Dyflos (Boots) was made up as a stock solution of 200 mg/ml in dried propylene glycol and kept at 0° C. For each experiment the solution was freshly diluted with artificial CSF. Atropine methyl nitrate (Sigma); carbachol (Savory & Moore); glycine (British Drug Houses); obidoxime [*N,N'*-oxydimethylene - bis - (pyridinium - 4 - aldoxime)-dichloride] (Toxogonin, Merck); pralidoxime mesylate (2-hydroxyiminomethyl-*N*-methyl pyridinium methyl methane-sulphonate) (Aldrich Chem. Co.); compound 30 [4-hydroxyiminomethyl-l-(3-*N,N*-dimethylaminopropyl) pyridinium chloride hydrochloride] was prepared as described by Ashani & Cohen (1967) and kindly provided by these authors; pilocarpine hydrochloride (Hopkin & Williams). All drugs were dissolved in artificial CSF prepared according to the formula given by Merlis (1940). All values given in the text refer to the salts.

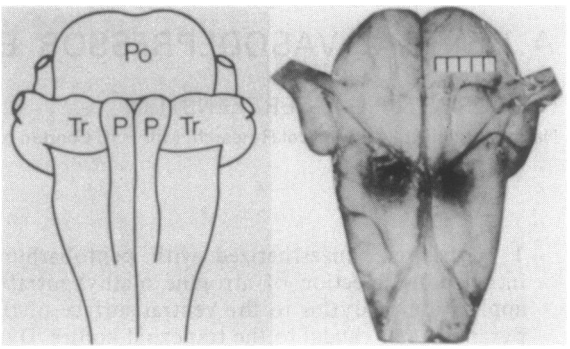


**Fig. 1** Diagram and photograph of the ventral surface of the brain stem of the cat. The dark areas in the photograph are due to staining by bromophenol blue which had been placed inside the perspex rings *in vivo*. Po, Pons; Tr, Trapezoid bodies; P, Pyramids. The scale refers to mm.

## Results

*Dyflos*

When applied to the ventral surface of the brain stem, dyflos produced a fall in arterial blood pressure without a change in heart rate. The blood pressure fell throughout the 5 min application and continued to fall for a few minutes after the dyflos was washed out of the rings; it remained low for various lengths of time and then gradually recovered. The effect, which was not prevented by an intravenous injection of atropine methyl nitrate, 2 mg/kg, was always obtained with 10 or 20 mg/ml, often with 5 mg/ml, but rarely with 1 mg/ml. The topical application of a control solution of propylene glycol in a concentration of 207 mg/ml, the concentration in which 20 mg/ml of dyflos was dissolved, did not affect blood pressure.

In experiments without artificial ventilation, dyflos increased rate and depth of respiration and produced a dissociation of thoracic and abdominal respiration. The expiratory phase of the thorax became associated with strong contractions of the muscles of the abdominal wall. These respiratory effects persisted for about 10 min after the dyflos was washed out.

Figure 2 shows the depressor effect of a topical application for 5 min of 5 mg/ml of dyflos in a cat which had not received intravenous atropine methyl nitrate and was respiring spontaneously. The blood pressure fell nearly 50 mmHg within 7 min, and remained low during the following hour. Respiration increased from 40 to 66/min,

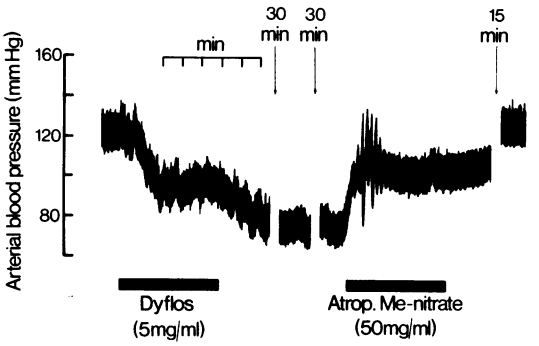


**Fig. 2** Record of arterial blood pressure from a 4.8 kg cat anaesthetized with i.p. pentobarbitone sodium. The black horizontal bars represent 5 min periods of bilateral application to the ventral surface of the brain stem of either dyflos (5 mg/ml) or atropine methyl nitrate (50 mg/ml). The breaks indicate intervals as stated.

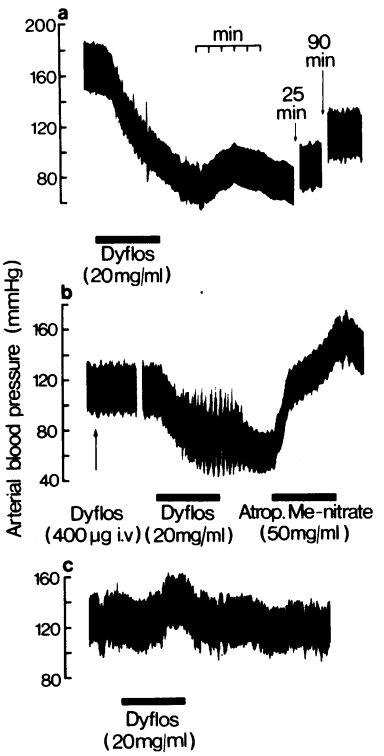


**Fig. 3** Records of arterial blood pressure from a 4.9 kg cat anaesthetized with i.p. pentobarbitone sodium, artificially ventilated and atropinized peripherally by intravenous injection of atropine methyl nitrate (2 mg/kg). The black horizontal bars below the records represent 5 min application to the ventral surface of the brain stem of either dyflos (20 mg/ml) or atropine methyl nitrate (50 mg/ml). At the arrow in record (b), intravenous injection of 400 µg dyflos. The breaks represent intervals as stated. Record (b) obtained 10 min after (a), record (c) 15 min after (b).

became deeper and showed a dissociation of thoracic and abdominal respiration.

Figure 3 shows the effect of dyflos in an artificially ventilated cat which had received intravenous atropine methyl nitrate. Figure 3a shows that the blood pressure fell about 90 mm during the 5 min of, and the 2 min following the application of 20 mg/ml of dyflos. The fall was then interrupted by a transient rise of about 20 mm. In some experiments the transient rise was more pronounced, in others it was absent. The record further illustrates the gradual nature of recovery. Blood pressure had not fully recovered 1.5 h after the dyflos had been washed out.

The depressor effect of dyflos was not due to its absorption into the blood stream. As the dyflos was put into each ring in a volume of 10 µl, both rings together contained 400 µg. If all the dyflos had been absorbed it would not have been sufficient to lower the blood pressure because an intravenous injection of 400 µg did not affect the blood pressure, as shown in Figure 3b. The cat had not become insensitive to dyflos, because when dyflos was subsequently applied topically it still produced a fall in blood pressure. In other experiments it was found that a slow intravenous infusion of 400 µg dyflos during 5 min also did not affect the blood pressure. Similar experiments were done on cats that had not been given atropine methyl nitrate intravenously. In one such experiment in which the topical application of 5 mg/ml of dyflos had lowered blood pressure by about 50 mm, an intravenous injection of 100 µg dyflos, the total amount placed into the rings, did not affect the blood pressure.

*Topical application of atropine methyl nitrate.* It was previously shown that atropine methyl nitrate placed in the perspex rings prevented and abolished the depressor effect of carbachol and physostigmine but not of glycine and γ-aminobutyric acid similarly applied (Guertzenstein, 1973). Atropine methyl nitrate, applied in this way, also antagonized the depressor effect of topically applied dyflos. This is shown in Fig. 2 for a cat without, and in Fig. 3b for a cat with a previous intravenous injection of atropine methyl nitrate. In both experiments the blood pressure which had fallen following the topical application of dyflos, began to rise immediately when atropine

methyl nitrate (50 mg/ml) was placed inside the perspex rings.

After a 5 min topical application of atropine methyl nitrate, the cat did not respond for at least 2 h with a fall in blood pressure to topical application of dyflos, which instead produced a small rise in pressure. This is shown in Figure 3c.

*Oximes*

*Obidoxime.* When applied to the ventral surface of the brain stem, obidoxime produced no changes in arterial blood pressure, or only a slight rise which did not become greater on increasing the obidoxime concentration. This is shown in Fig. 4a, which gives the effect of topical application of obidoxime, first in a concentration of 100 mg/ml and then of 200 mg/ml. However, when the blood pressure had fallen following the dyflos application, obidoxime, in a concentration of 100 mg/ml produced full, or nearly full reversal of the blood pressure fall. Such a reversal is illustrated in Figure 4b. A reversal did not occur on intravenous injection of 2 mg obidoxime, the amount placed in both rings, or even with double this amount.

The topical application of obidoxime also reversed the fall in blood pressure produced by topical application of carbachol, but not of glycine. The reversal of the fall produced by 6 mg/ml of carbachol is shown in Fig. 4c, whereas Fig. 4d shows that the fall produced by 100 mg/ml of glycine was not reversed by obidoxime.

*Pralidoxime mesylate.* When applied to the ventral surface of the brain stem pralidoxime mesylate acted like obidoxime. On its own it had either no effect on arterial blood pressure, or produced a small rise which did not become greater with an increase in concentration. When applied after the blood pressure had fallen following the topical application of either dyflos or carbachol it reversed the fall.

Figure 5a shows the effect of 100 and 200 mg/ml of pralidoxime mesylate in a cat which had not been treated with dyflos. Figure 5b shows partial reversal obtained in another cat with 100 mg/ml applied during a fall in blood pressure produced by dyflos. Weight for weight, pralidoxime mesylate was less potent than obidoxime. From a comparison of Fig. 5b with Fig. 4b, it is evident that with pralidoxime the blood pressure rose more gradually than on application of 100 mg/ml of obidoxime which produced nearly full reversal. To obtain full, or nearly full reversal with pralidoxime it had to be applied in a concentration of 200 mg/ml. The effect of pralidoxime was not due to absorption into the blood

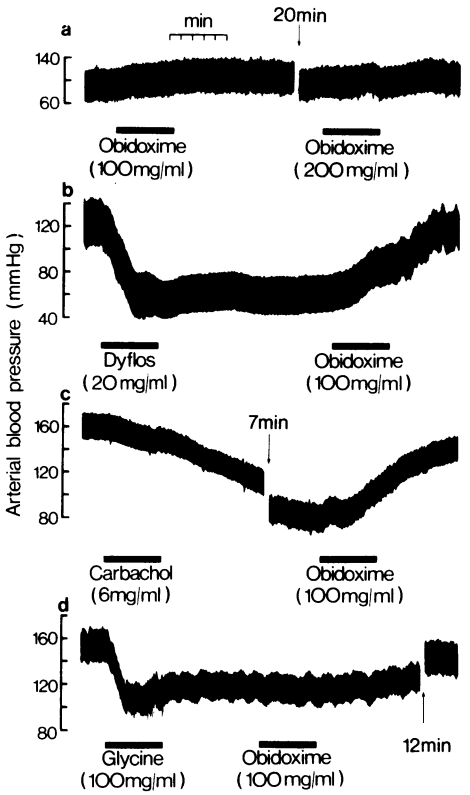


**Fig. 4** Records of arterial blood pressure from four cats anaesthetized with i.p. pentobarbitone sodium, artificially ventilated and atropinized peripherally by intravenous injection of atropine methyl nitrate (2 mg/kg). The black horizontal bars represent 5 min periods of application to the ventral surface of the brain of dyflos, obidoxime, carbachol or glycine in the concentrations indicated. The breaks indicate intervals as stated. Record (a) from a 4 kg, (b) from a 3.9 kg, (c) from a 4.2 kg, and (d) from a 3.9 kg cat.

stream because no reversal occurred on its intravenous injection in doses of 2 or 4 mg.

Figure 5c and d, show the effect of pralidoxime on the fall in blood pressure produced by topical application of 6 mg/ml of carbachol in another cat. The fall was partly reversed by the topical application of 100 mg/ml, and with subsequent application of 200 mg/ml full recovery occurred. The blood pressure fall produced by topical application of glycine (100 mg/ml) was not affected by pralidoxime mesylate similarly applied.

*Compound 30.* This reactivator of acetylcholinesterase inhibited by organophosphates (Edery (1970); Edery, Soroker & Kuhnberg (1970)) did

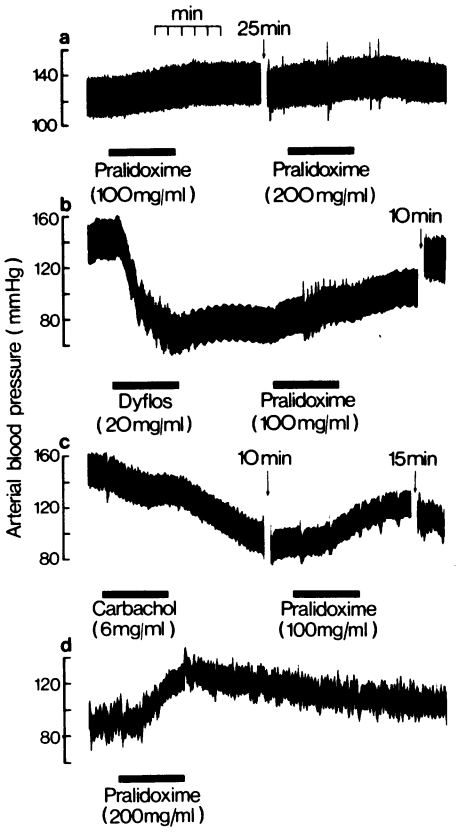


**Fig. 5** Records of arterial blood pressure from three cats anaesthetized with i.p. pentobarbitone sodium, artificially ventilated and atropinized peripherally by intravenous injection of atropine methyl nitrate (2 mg/kg). The horizontal bars represent 5 min periods of application to the ventral surface of the brain stem of pralidoxime mesylate, dyflos or carbachol, in the concentrations indicated. The breaks indicate intervals as stated. Record A from a 3.7 kg, B from a 4.2 kg, C and D from a 3.8 kg cat. Interval between C and D, 2 minutes. Arterial blood pressure in mmHg. Time in minutes.

not act like obidoxime or pralidoxime mesylate. Applied in a concentration of 100 mg/ml to the ventral surface of the brain stem it did not affect the blood pressure when it had fallen as a result of a previous application of either dyflos (20 mg/ml) or carbachol (6 mg/ml). When given alone, compound 30 sometimes produced a small fall in blood pressure.

*Pilocarpine*

This parasympathomimetic substance did not have the vasodepressor effect of carbachol, physostigmine and dyflos, when applied to the ventral surface of the brain stem. In a concentration of 100 or 200 mg/ml, pilocarpine had no effect on arterial blood pressure, and in cats not artificially ventilated, the rate of respiration did not change.

## Discussion

Dyflos shares with physostigmine, carbachol, glycine, γ-aminobutyric acid (Guertzenstein, 1973), pentobarbitone sodium (Feldberg & Guertzenstein, 1972) and clonidine (Bousquet & Guertzenstein, 1973), the property of lowering arterial blood pressure when applied bilaterally to the ventral surface of the brain stem at a region lateral to the pyramids and caudal to the trapezoid bodies. This does not imply that all these substances act on the same synapses in this region, although as far as the cholinomimetic substances are concerned, the same cholinoceptive cells may be affected. The fall in blood pressure produced by dyflos is attributed to a decrease in sympathetic vasomotor tone since it occurred after intravenous injection of atropine methyl nitrate which does not readily pass the blood brain barrier, and since it was not associated with bradycardia.

The finding that dyflos produced an increase in frequency and depth of respiration when applied to the ventral surface of the brain stem is in accord with the observation by Mitchell, Loeschcke, Severinghaus, Richardson & Massion (1963) that acetylcholine had this action when similarly applied, although the region from where the acetylcholine effect was obtained extended more rostrally than the area covered by the perspex rings in the present experiments. The dissociation produced by dyflos of thoracic and abdominal respiration, in that the expiratory phase of the thorax was associated with contractions of the abdominal muscles, may be attributed to indiscriminate stimulation by dyflos of expiratory and inspiratory structures (or centres). Trouth, Loeschcke & Berndt (1973), showed with point-to-point electrical stimulation of the ventral surface of the medulla oblongata that expiratory and inspiratory effects could be obtained from different regions which, however, were lying close to each other. They mapped out regions which contained only expiratory or only inspiratory structures and those which contained both structures. The main regions in which these different structures were located would be covered by the perspex rings through which the dyflos was applied in the present experiments.

Of the three oximes examined, only two, obidoxime and pralidoxime were found to reverse

the dyflos-induced fall in blood pressure when applied to the ventral surface of the brain stem, whereas compound 30 did not do so. Yet all three oximes reactivate acetylcholinesterase inhibited by organophosphates, the most potent being obidoxime (Hobbiger, 1963; Erdmann & Engelhard, 1964; Hobbiger & Vojvodić, 1966; Edery *et al.*, 1970).

Another possibility is that the reversal of the dyflos-induced fall in blood pressure by obidoxime and pralidoxime was due to their central atropine-like action. This possibility had to be considered because in higher concentrations both oxidoxime and pralidoxime have a peripheral atropine-like action and antagonize the contractions produced by acetylcholine and carbachol on isolated smooth muscle preparations (Bethe, Erdmann, Lendle & Schmidt, 1957; Hobbiger & Sadler, 1959; Lehmann, 1962; Kuhnen-Clausen, 1970). They may therefore also have a central atropine-like action. An atropine-like action would explain why compound 30 did not reverse the dyflos-induced fall in blood pressure because this reactivator has no peripheral atropine-like action as it does not antagonize the acetylcholine-induced contractions of the guinea-pig ileum preparation (Edery *et al.*, 1970). It would therefore probably also lack a central atropine-like action. The small rise in blood pressure sometimes produced by obidoxime and pralidoxime when applied without prior application of dyflos to the ventral surface of the brain stem could also be explained by an atropine-like effect since atropine itself produced a small rise in blood pressure when applied in this way (Guertzenstein, 1973).

In order to obtain more information on this point, the effect of the oximes was examined on the vasodepression produced when carbachol was applied to the ventral surface of the brain stem. Carbachol is an agonist on cholinoreceptors but is known also to carbamylate the acetylcholinesterase (Wilson, Harrison & Ginsburg, 1961; Reiner & Aldridge, 1967). This mode of action must be considered when a high concentration of carbachol is required to produce an effect. With the method of topical application of drugs to the ventral surface of the brain stem they have to be applied in high concentrations and the vasodepressor effect of carbachol was obtained on its application in a concentration of 6 mg/ml. The vasodepression could therefore have resulted from carbamylation of acetylcholinesterase, and consequently the reversal of the blood pressure fall by obidoxime and pralidoxime from reactivation of the enzyme by decarbamylation. This interpretation, however, would not explain why compound 30 did not reverse the carbachol vasodepression. Further, the type of carbamylated acetylcholinesterase readily undergoes spontaneous reactivation and there is no experimental evidence in the literature of effective decarbamylation of the enzyme by quaternary pyridinium aldoximes *in vivo*. Nevertheless, this possibility cannot be excluded with certainty. It is therefore not possible to state definitely whether the reversal of the dyflos- and carbachol-induced vasodepression by obidoxime and pralidoxime is due to reactivation of acetylcholinesterase by dephosphorylation or decarbamylation respectively, or to a central atropine-like action, or to a combination of the two effects.

Pilocarpine did not produce a fall in arterial blood pressure when applied to the ventral surface of the brain stem and thus differed from dyflos, physostigmine and carbachol. This is not the only instance in which pilocarpine does not reproduce central actions of cholinomimetic substances. In cats, Miller, Stavraky & Woonton (1940) found changes in the electrocorticogram with physostigmine topically applied to the cerebral cortex, but not with pilocarpine. More recently, Borison, Haranath & McCarthy (1972) described differences in the effect of cholinomimetic substances on their perfusion through the cerebral ventricles of cats. Changes in respiration and blood pressure occurred with acetylcholine and methacholine, but not with pilocarpine.

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