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Acetylcholinesterase-independent action of diisopropyl-flurophosphate in the rat aorta

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Abstract

Recent studies have shown that many organophosphates can bind competitively and noncompetitively to membrane muscarinic receptors. The present study investigated the responses of the rat aortic rings to diisopropyl-flurophosphate (DFP), an organophsophorus cholinesterase inhibitor, and the possible involvement of muscarinic receptors. DFP caused a concentration-dependent contraction when added cumulatively from 10^{-8} to 10^{-4} M. This contraction was inhibited in a noncompetitive manner by high concentrations of atropine (1.5×10^{-6} and 1.8×10^{-6} M) but was unaffected by similar concentrations of selective muscarinic receptor subtype antagonists, pirenzepine, 11-2[2-[(diethylamino)methyl]-1-piperidinyl]acetyl-5,11-dihydro-6*H*-rido[2,3-*b*][1,4]benzodiazepin-6-one (AF-DX116) and 4-Diphenylacetoxy-*N*-methyl piperidine methiodide (4-DAMP). Phentolamine, an α-adrenoceptor antagonist, was able to inhibit the DFP-induced contraction in a noncompetitive manner at a concentration of 10^{-7} M. These findings suggested that the DFP-induced contraction in the rat aortic rings was mediated by norepinephrine that was released from sympathetic nerve terminals present in the aortic rings.

Keywords: Organophosphate; Diisopropyl-flurophosphate; Aorta; (Rat); Phentolamine; Norepinephrine

1. Introduction

The organophosphate group of compounds is broad and includes insecticides, such as DFP (diisopropyl-flurophosphate), parathion (*O*,*O*-diethyl-*O*-(4-nitrophenyl)-phosphorothiolate) and the highly toxic nerve gases VX (ethyl-*S*-2 *N*,*N*-diisopropylaminoethylmethylphosphonofluoridate), sarin (isopropylmethylphosphono-fluoridate), soman (pinacolyl methylphosphonofluoridate) and tabun (ethyl-*N*,*N*-dimethyl-phosphoramodocyanidate). These nerve agents present a major threat to soldiers on battlefields and occasionally have been used by terrorists against civilians (Gunderso et al., 1992; Solberg and Belking, 1997). The toxic effects of the organophosphates are due to their ability to inhibit cholinesterase. However, many organophosphates have been shown to bind competitively (Bakry et al., 1988; Silveira et al., 1990) and noncompetitively (Katz and Marquis, 1989) to membrane muscarinic receptors. Ward et al. (1993) demonstrated that DFP, a potent organophosphate, could interact directly with muscarinic receptors and competitively inhibit the binding of [^{3}H]cis-methyldioxolane (muscarinic M_2 receptor subtype agonist) to muscarinic receptors in the hippocampus and frontal cortex of rats. Possible functional responses of DFP, arising from its binding to peripheral vascular muscarinic receptors, may alter vascular contractility and exacerbate organophosphate-induced hypertension (Maxwell et al., 1987; Bataillard et al., 1990; Kassa and Fusek, 1997). As there is little information available on the functional consequences of exposure to DFP, the aim of this study was to investigate the possible effects of DFP on peripheral vascular tissue, namely the rat aorta, using a functional organ bath system.

The results from this study suggest that DFP caused contraction via the release of norepinephrine from adrenergic nerve endings. This finding may help to explain the cardiovascular effects seen in organophosphate intoxication, namely peripheral vasoconstriction and the reduction of blood flow through detoxifying organs (Maxwell et al., 1987).

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2. Materials and methods

2.1. Preparation of aortic rings

Aortae were obtained from male Sprague–Dawley rats (300–350 g) that had been paralysed by cervical dislocation. Ring preparations from the aortae were made, as described previously by Sim and Singh (1987), in Krebs–Ringer bicarbonate buffer containing (in mM) 113 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2 \cdot 7H_2O$, 20 $NaHCO_3$, 10 glucose and 0.26 EDTA (as an antioxidant of norepinephrine, Maxwell et al., 1983), pH 7.4, and equilibrated with 95% O_2: 5% CO_2. Each ring was then suspended in a 10-ml organ bath containing Krebs–Ringer bicarbonate buffer with one end connected by a silk thread to a force-displacement transducer. The Krebs–Ringer was maintained at 37°C and continuously equilibrated with 95% O_2: 5% CO_2.

The rings were equilibrated for 60 min under 1 g tension and the bathing fluid was changed every 15 min. To test the endothelium intactness of each ring, 10^{-7} M norepinephrine was added to produce a contraction (80% of the maximum response to the catecholamine). The norepinephrine-contracted ring was then relaxed with 2×10^{-6} M acetylcholine. Rings that did not relax by more than 80% were discarded.

2.2. Effects of DFP on endothelium-intact and endothelium-denuded aortic rings

Contractions in response to DFP were determined by the cumulative addition of 10^{-8}–10^{-4} M DFP to the aortic rings. Changes in tension are expressed as percentages of the tension generated in response to 10^{-7} M norepinephrine. The experiments were repeated with endothelium-denuded rings that were prepared by gently rubbing the aortic lumen with the blunted tip of a glass pipette. Properly denuded aortic rings precontracted with 10^{-7} M norepinephrine would be unresponsive to relaxation by 2×10^{-6} M acetylcholine.

2.3. Effects of repeated DFP exposure on the response of aortic rings

Aortic rings were exposed to cumulative concentrations of DFP (10^{-8}–10^{-4} M), washed and equilibrated for 90 min and reexposed to the same cumulative range of DFP concentratons. The aortic rings were, subsequently, washed and equilibrated for another 90 min and contracted with 10^{-7} M norepinephrine. The last procedure was used to determine the deterioration of contractility of the rings.

2.4. Effects of pirenzepine, 4-diphenylacetoxy-N-methyl piperidine methiodide (4-DAMP) and atropine on DFP-induced contractions

Muscarinic antagonists were used to study the likely mechanism of DFP actions. As the response to DFP showed tachyphylaxis, the following procedure was adopted. Aortic rings were preincubated with a different concentration of antagonist for 30 min and then exposed to cumulative concentrations of DFP. Each ring was exposed to only one concentration of the antagonist and a set of cumulative concentrations of DFP. The experiments were carried out using muscarinic subtype specific antagonists, pirenzepine, 11-2[2-[(diethylamino)methyl]-1-piperidinyl]acetyl-5,11-dihydro-6*H*-rido[2,3-*b*][1,4]benzodiazepin-6-one (AFDX 116), 4-DAMP and atropine. Control responses were obtained from rings that were not exposed to the antagonists.

2.5. Effects of phentolamine on DFP-induced contractions

The possible involvement of adrenoceptors was investigated using phentolamine, an α-adrenoceptor antagonist. The procedure was as described for the study of muscarinic antagonists.

2.6. Drugs

DFP, atropine sulphate, phentolamine and pirenzepine were purchased from Sigma (St. Louis, MO). AF-DX116 and 4-DAMP were generous gifts from Boeringer-Ingelheim and Dr. R.B. Barlow (University of Bristol, UK), respectively.

2.7. Statistics and analysis of data

The values given in the text and figures are means ± S.E.M. Data were compared statistically using nonparametric Mann–Whitney Test and comparisons with P values < 0.05 were considered to be significant. Concentrations of DFP that produced 50% of the maximum response (EC_{50}) were calculated by linear regression of all points between 20% and 80% of the maximum response to norepinephrine. The EC_{50} values were used to calculate the shift in the concentration–response curves of preparations treated with receptor antagonists.

3. Results

3.1. Effects of DFP on endothelium-intact and endothelium-denuded aortic rings

Exposure of endothelium-intact aortic rings to cumulative concentrations of DFP caused a progressive increase in contractile response (Fig. 1). The maximum contraction occurred at 10^{-4} M DFP and constituted 44 ± 1.6 % of the maximum response produced by 10^{-7} M norepinephrine (Fig. 1). Endothelium-denuded aortic rings responded in a similar manner and responses were not significantly different from those of endothelium-intact rings.

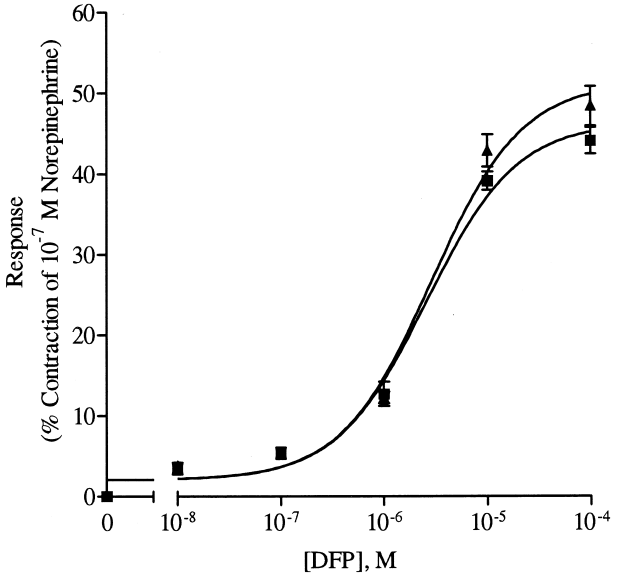

Fig. 1. Response of endothelium-intact (■) and -denuded (▲) aortic rings to cumulative concentrations of DFP. Thoracic aortas were prepared and pretreated as described in Materials and methods. Each point is the mean ± S.E.M. of eight observations obtained from eight individual rats.

3.2. Effects of repeated DFP exposure on the response of aortic rings

Fig. 2. shows the responses of aortic rings to repeated cumulative exposure to DFP. Maximum responses of the rings to repeated DFP exposure were significantly reduced by 68% when compared to those of the first exposure (Fig. 2).

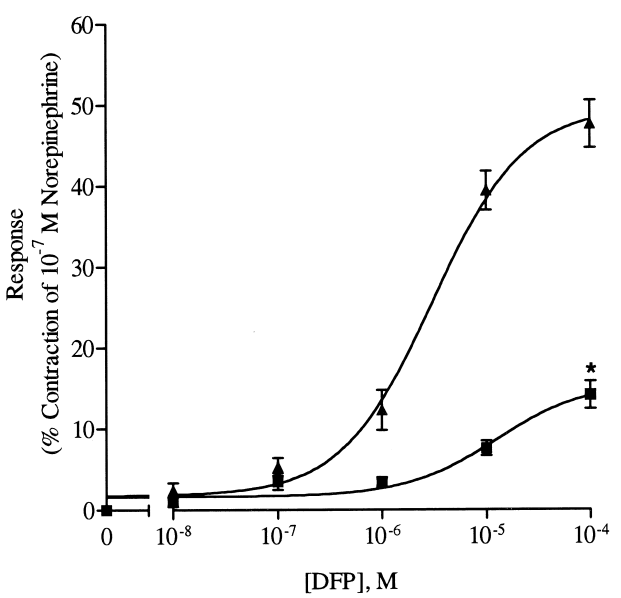

Fig. 2. Response of endothelium-intact aortic ring to first (▲) and second (■) cumulative concentrations of DFP. Thoracic aorta were prepared and pretreated as described in Materials and methods. Each point is the mean ± S.E.M. of five observations obtained from five individual rats.

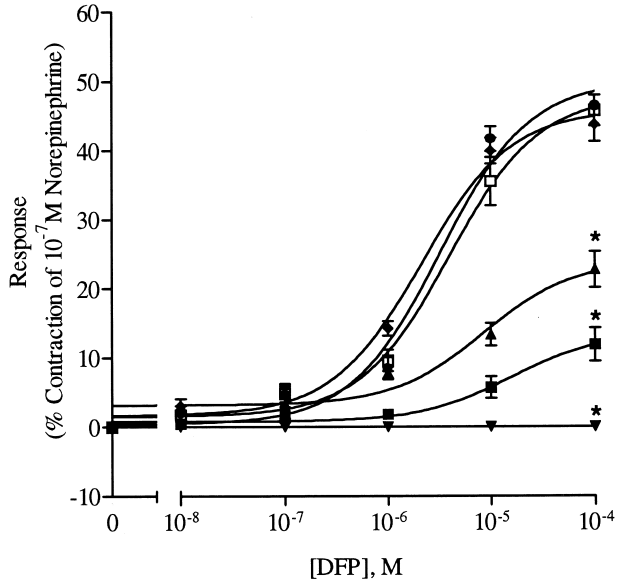

Fig. 3. Effects of atropine on DFP-induced contractions in rat thoracic aorta. Aortic rings were prepared and pretreated as described in Materials and methods. Aortic rings were pretreated with different concentrations of atropine (● = 10^{-7} M; ◆ = 10^{-6} M; ▲ = 1.5×10^{-6} M; ■ = 1.8×10^{-6} M; ▼ = 10^{-5} M of atropine, respectively). Control response (□) was obtained from rings that were not treated with atropine. Each point is the mean ± S.E.M. of five observations obtained from five individual rats.

3.3. Effects of atropine on DFP-induced contractions

Aortic rings pretreated with 10^{-7} and 10^{-6} M atropine showed no significant differences in maximum responses compared to control rings (Fig. 3). Significant reductions of the maximum responses were observed when concentrations of more than 10^{-6} M were used (Fig. 3). At 1.5×10^{-6} M, atropine caused a significant reduction of the maximum response by 50% (Fig. 3) and significantly shifted the concentration–response curve (2.5-fold) to the right. With a higher concentration of 1.8×10^{-6} M, atropine caused the maximum responses of rings to be further reduced by a significant 74% (Fig. 3) and shifted the concentration–response curve significantly to the right by fourfold. At 10^{-5} M, atropine completely abolished the response to DFP (Fig. 3).

3.4. Effects of pirenzepine, AF-DX116 and 4-DAMP on DFP-induced contractions

Pretreatment with muscarinic subtype specific antagonists pirenzepine (Fig. 4), AF-DX116 (Fig. 5) and 4-DAMP (Fig. 6) had no significant effect on the concentration–response curves for DFP.

3.5. Effects of phentolamine on DFP-induced contractions

Aortic rings pretreated with 10^{-9} and 10^{-8} M phentolamine showed no significant differences in maximum

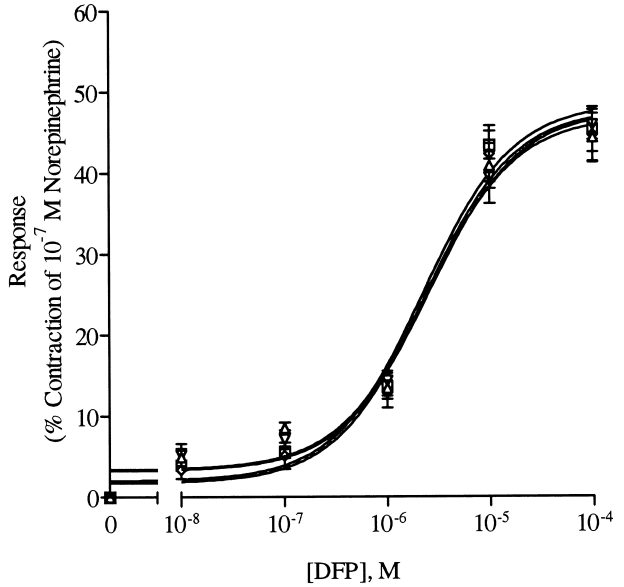

Fig. 4. Effects of pirenzepine on DFP-induced contractions in rat thoracic aorta. Aortic rings were prepared and pretreated as described in Materials and methods. Aortic rings were pretreated with different concentrations of pirenzepine (□ $=10^{-7}$ M; △ $=10^{-6}$ M; ▽ $=10^{-5}$ M of pirenzepine, respectively). Control response (◇) was obtained from rings that were not treated with pirenzepine. Each point is the mean ± S.E.M. of five observations obtained from five individual rats.

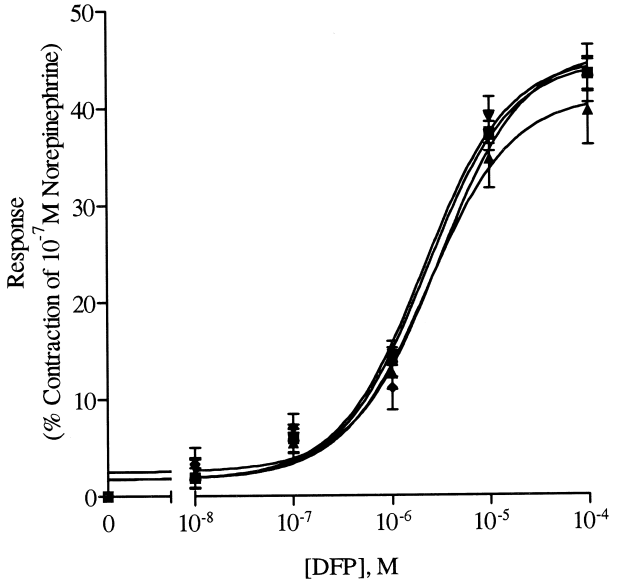

Fig. 6. Effects of 4-DAMP on DFP-induced contractions in rat thoracic aorta. Aortic rings were prepared and pretreated as described in Materials and methods. Aortic rings were pretreated with different concentrations of 4-DAMP (■ $=10^{-7}$ M; ▲ $=10^{-6}$ M; ▼ $=10^{-5}$ M of 4-DAMP, respectively). Control response (◆) was obtained from rings that were not treated with 4-DAMP. Each point is the mean ± S.E.M. of five observations obtained from five individual rats.

responses when compared to control rings (Fig. 7). When rings were pretreated with 1.5×10^{-8} M phentolamine, maximum contractile responses to DFP were significantly reduced by 76% (Fig. 7) and the concentration response curve was significantly shifted 7.6-fold to the right. 10^{-7} M phentolamine completely abolished contractions induced by DFP.

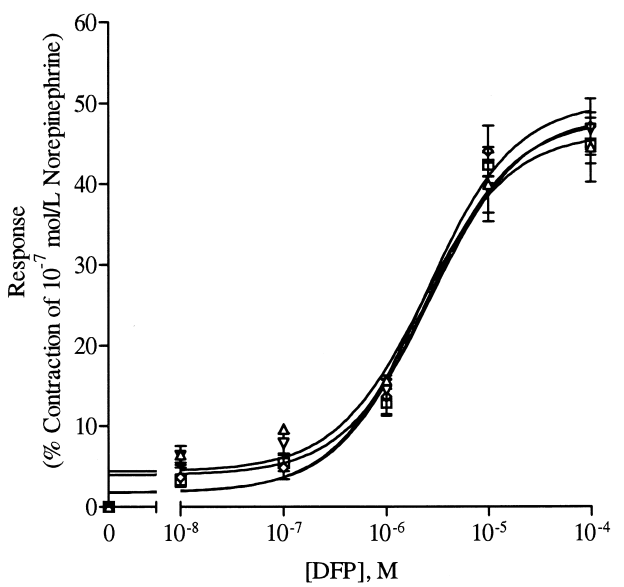

Fig. 5. Effects of AF-DX116 on DFP-induced contractions in rat thoracic aorta. Aortic rings were prepared and pretreated as described in Materials and methods. Aortic rings were pretreated with different concentrations of AF-DX116 (□ $=10^{-7}$ M; △ $=10^{-6}$ M; ▽ $=10^{-5}$ M of AF-DX116, respectively). Control response (◇) was obtained from rings that were not treated with AF-DX116. Each point is the mean ± S.E.M. of five observations obtained from five individual rats.

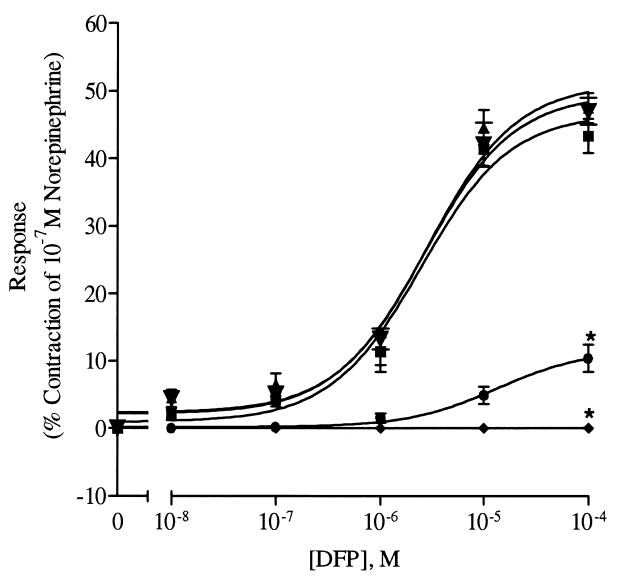

Fig. 7. Effects of phentolamine on DFP-induced contractions in rat thoracic aorta. Aortic rings were prepared and pretreated with different concentrations of phentolamine (▲ $=10^{-9}$ M; ▼ $=10^{-8}$ M; ● $=1.5\times 10^{-8}$ M; ◆ $=10^{-7}$ M of phentolamine) as described in Materials and methods. Aortic rings were pretreated . Control response (■) was obtained from rings that were not treated with phentolamine. Each point is the mean ± S.E.M. of six observations obtained from six individual rats.

4. Discussion

The present investigation demonstrated that DFP could induce concentration-dependent contractions in rat aortic rings. Since the anticholinesterase action of DFP would result in accumulation of unhydrolyzed acetylcholine, it was important to determine whether acetylcholine was the mediator of the contractile effects observed with DFP. Eglen and Whiting (1990) reported that acetylcholine could contract and relax vascular tissue, with the latter effect mediated by the release of relaxant factors, such as nitric oxide via the activation of endothelial muscarinic M_3 receptors (Eglen et al., 1996). If the contractile effect induced by DFP was indeed mediated by acetylcholine, DFP-induced concentration-dependent contractions would be potentiated by the removal of the endothelium from aortic rings. However, this was not observed with endothelium-denuded preparations, which indicated that the contractile effects of DFP were not mediated by acetylcholine. Moreover, most blood vessels, including the aorta, do not receive parasympathetic innervation (Hoffman et al., 1996) and, therefore, lack the acetylcholine source.

The muscarinic receptors mediating contraction of vascular tissue differ according to species and anatomical location. Muscarinic M_3 subtype receptors mediate contractions of rat coronary vascular bed (Su and Narayanan, 1993), the spontaneously hypertensive rat aorta (Boulanger et al., 1994), the rat aorta (Watson and Eglen, 1994), the simian coronary artery (Obi et al., 1995) and human isolated pulmonary arteries (Norel et al., 1996). This receptor subtype also mediates contraction of guinea pig isolated portal vein (Pfaffendorf and Van Zwieten, 1993). The muscarinic M_1 receptor subtype was reported to mediate the contraction in rabbit pulmonary vessels (El-Kashef and Catravas, 1991), canine isolated femoral and saphenous veins and cat cerebral arteries (O'Rourke and Vanhoutte, 1987; Eglen et al., 1990; Dauphin and Hamel, 1992). More recently, Nasa et al. (1997) suggested that the muscarinic M_2 receptor subtype played a role in acetylcholine-induced vasoconstrictor response in rat coronary vessels. To determine whether muscarinic receptors played a role in DFP-induced contractions, competitive muscarinic antagonists, atropine, pirenzepine, AF-DX116 and 4-DAMP, were used. Pretreatment with 10^{-7} and 10^{-6} M atropine had no effect on the DFP response curves. However, when higher concentrations of atropine (1.5×10^{-6} and 1.8×10^{-6} M) were used, dose-dependent significant reductions in maximum responses and rightward shifts in the concentration–response curves were observed. The inhibition by high concentrations of atropine coupled with the noncompetitive antagonism observed (decrease in maximum responses) raised doubts as to whether it was the result of muscarinic receptor antagonism. Atropine is a potent and competitive muscarinic antagonist. It inhibits at submicromolar concentrations and with a reversible maximum response (Goyal, 1989; Levine and Birdsall, 1989). The absence of effects elicited by pirenzepine (muscarinic M_1 subtype specific), AF-DX116 (muscarinic M_2 subtype specific) and 4-DAMP (muscarinic M_3 subtype specific) also suggested that the observed inhibition was probably atropine, but not muscarinic-specific.

Day (1967) showed that pressor responses to indirectly acting sympathomimetic amines, dexamphetamine and phenylethylamine, demonstrated tachyphylaxis. He attributed the tachyphylaxis to possible exhaustion of vesicles in the sympathetic neurones. Consistent with this phenomenon was our finding of tachyphylatic responses of aortic rings to DFP. Repeated cumulative exposure to DFP resulted in marked reduction of the contractile responses to DFP. Since the contractile responses to DFP showed marked tachyphylaxis while those to norepinephrine were unaffected, it was unlikely that the deterioration of the contractility of the rings was the cause of the decrease in maximum contractile responses induced by DFP.

Nedergaard and Schrold (1977) showed that atropine at high concentrations could also bind nonspecifically to postsynaptic α-adrenoceptors. Since our findings showed that DFP-induced contractile effects could only be inhibited by high concentrations of atropine, the possible participation of α-adrenoceptors cannot be discounted. This hypothesis was investigated using phentolamine, a competitive α-adrenoceptor antagonist. Unlike atropine, phentolamine significantly reduced DFP-induced contractions at 1.5×10^{-8} M, a dose that is 100-fold lower than the former. This finding suggests that the inhibitory action of DFP is probably α-adrenoceptor-specific.

Kenakin (1987) reported that if a competitive antagonist depressed the maximum response to an agonist with an unknown mechanism of actions, then the release of an endogenous agonist (i.e. neurotransmitter) was implicated. The depression of maximum responses is atypical of atropine and phentolamine as competitive antagonists. These observations, however, were reminiscent of the depression of the maximum response to the indirect agonist tyramine by propranolol in the rat heart (Black et al., 1980). Tyramine produced a response by initiating the release of norepinephrine from adrenergic nerve endings that innervate cardiac tissue (Trendelenburg, 1972). Consistent with this, Nedergaard and Schrold (1977) also reported that atropine inhibited the tyramine-induced contractions in isolated rabbit pulmonary artery. In view of the similar observations with atropine, it is likely that DFP could act as an indirect agonist and induce contractions by initiating the release of norepinephrine from adrenergic nerve endings innervating the aortic vascular wall. This would explain the depression of maximum responses elicited by phentolamine in the DFP concentration–response curves.

The present results do not allow any conclusion with respect to the mechanism of release of norepinephrine by DFP, but they support earlier findings by Chao et al. (1988), who demonstrated the ability of organophosphates to release intraneuronal norepinephrine in vascular tissue.

Their findings showed that soman, a potent organophosphorus compound, could initiate the release of norepinephrine from intraneuronal stores. Norepinephrine causes contractions in almost all blood vessels that are innervated by adrenergic nerves (Langer and Hicks, 1984). Hence, if DFP could initiate the release of norepinephrine from adrenergic nerves, it might have the potential to alter blood pressure and local blood flow. The present findings may explain, in part, the cardiovascular effects of organophosphate intoxication manifested as peripheral vasoconstriction (Stewart and McKay, 1961; Kentera et al., 1986) and reduction of blood flow through detoxifying organs (Maxwell et al., 1987). Because the concentration of DFP used was much higher than was necessary to inhibit cholinesterase activity, DFP might have other effects on the cells in the aorta, such as effects on ion channels. The ion channels present both pre- and post-synaptically could be affected. Of particular interest was the observation showing the interaction of DFP with presynaptic glutamate receptors, which resulted in transmitter release, leading to spontaneous excitatory postsynaptic potentials and muscle action potentials (Idriss et al., 1986). A study with ion-channel blockers to identify the possible ion channels involved in the DFP-induced release of norepinephrine is ongoing in our laboratory.

Acknowledgements

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References

Bakry, N.M.S., El-Rashidy, A.H., Eldefrawi, A.T., Eldefrawi, M.E., 1988. Direct actions of anticholinesterase on nicotinic and muscarinic acetylcholine receptors. J. Biochem. Toxicol. 3, 235–259.

Bataillard, A., Sannajust, F., Yoccox, D., Blanchet, G., Sentenac-Roumanou, H., 1990. Cardiovascular consequences of organophosphorus poisoning and of antidotes in conscious unrestrained rats. Pharmacol. Toxicol. 67, 27–35.

Black, J.W., Jenkinson, D.H., Kenakin, T.P., 1980. Antagonism of an indirectly acting agonist; block by propranolol and sotalol of the action of tyramines on rat heart. Eur. J. Pharmacol. 65, 1–10.

Boulanger, C.M., Morrison, K.J., Vanhoutte, P.M., 1994. Mediation by M_3 muscarinic receptors of both endothelium-dependent contraction and relaxation to acetylcholine in the aorta of spontaneously hypertensive rats. Br. J. Pharmacol. 112, 519–524.

Chao, Y.H., Chia, Y.H., Robinson, C.P., 1988. Effects of soman on vascular contractility of rabbit arteries. J. Toxicol. Environ. Health 24, 201–215.

Dauphin, F., Hamel, E., 1992. Identification of multiple muscarinic binding site subtypes in cat and human cerebral vasculature. J. Pharmacol. Exp. Ther. 260, 660–667.

Day, M.D., 1967. The lack of cross tachyphylaxis between tyramine and some other indirectly acting sympathomimetic amines. Br. J. Pharmacol. Chemother. 17, 245–260.

Eglen, R.M., Whiting, R.L., 1990. Heterogenity of vascular muscarinic receptors. J. Auton. Pharmacol. 19, 233–245.

Eglen, R.M., Michel, A.D., Montgomery, W.W., Kunysz, E.A., Machado, C.A., Whiting, R.L., 1990. The interaction of parafluorohexahydrosiladifenidol at muscarinic receptors in vitro. Br. J. Pharmacol. 99, 637–642.

Eglen, R.M., Hegde, S.S., Watson, N., 1996. Muscarinic receptor subtypes and smooth muscle function. Pharmacol. Rev. 48 (4), 531–565.

El-Kashef, H., Catravas, J.D., 1991. The nature of the muscarinic receptor subtypes mediaiting pulmonary vasoconstriction in the rabbit. Pulm. Pharmacol. 4, 8–19.

Goyal, R.K., 1989. Muscarinic receptor subtypes. Physiology and clinical implications. N. Engl. J. Med. 321, 1022–1029.

Gunderso, C.H., Lehman, C.R., Sidell, F.R., Jabbari, B., 1992. Nerve agents: a review. Neurology 42, 946–950.

Hoffman, B.B., Lefkowitz, R.J., Taylor, P., 1996. Neurotransmission: the autonomic and somatic nervous systems. In: Molinoff, P.B., Ruddon, R.W. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th edn. McGraw-Hill, New York, NY, pp. 105–139.

Idriss, M., Aguayo, L.G., Rickett, D.L., Albuquerque, E.X., 1986. Organophosphate and carbamate compounds have pre- and post-junctional effects at the insect glutamatergic synapse. J. Pharmacol. Exp. Ther. 239, 279–285.

Kassa, J., Fusek, J., 1997. Effects of panpal pretreatment and antidotal treatment (HI-6 plus benactyzine) on respiratory and circulatory function in soman-poisoned rats. Hum. Exp. Toxicol. 16, 563–569.

Katz, L.S., Marquis, J.K., 1989. Modulation of central muscarinic receptor binding in vitro by ultralow levels of the organophosphate paraoxon. Toxicol. Appl. Pharmacol. 101, 114–123.

Kenakin, T.P., 1987. Drug antagonism. In: Kenakin, T.P. (Ed.), Pharmacologic Analysis of Drug–Receptor Interaction. Raven Press, New York, NY, pp. 205–244.

Kentera, D., Susie, D., Stamenkovic, B., 1986. The effects of HS-3 and HS-6 on cardiovascular changes in rats by soman. Arh. Hig. Rada. Toksikol. 33, 143–150.

Langer, S.Z., Hicks, P.E., 1984. Alpha-adrenoceptor subtypes in blood vessels: physiology and pharmacology. J. Cardiovasc. Pharmacol. 6, S547–S558.

Levine, R.M., Birdsall, N.J.M., 1989. Nomenclature for muscarinic receptor subtypes recommended by symposium. Trends Pharmacol. Sci. S10 (VII).

Maxwell, L.C., Herlihy, J.T., Riedel, G.L., 1983. Effects of ascorbic acid and EDTA on vascular concentration–response to catecholamines. Microvasc. Res. 26, 81–88.

Maxwell, D.H., Lenz, D.E., Groff, W.A., Kaminskis, A., Froelich, H.L., 1987. The effects of blood flow and detoxification on in vivo cholinesterase inhibition by soman in rats. Toxicol. Appl. Pharmacol. 88, 66–76.

Nasa, Y., Kume, H., Takeo, S., 1997. Acetylcholine-induced vasoconstrictor response of coronary vessels in rats: a possible contribution of muscarinic M_2 receptor activation. Heart Vessels 12 (4), 179–191.

Nedergaard, O.A., Schrold, J., 1977. Effect of atropine on vascular adrenergic neuroeffector transmission. Blood Vessels 14, 325–347.

Norel, X., Walch, L., Costantino, M., Labat, C., Gorenne, I., Dulmet, E., Rossi, F., Brink, C., 1996. M_1 and M_3 muscarinic receptors in human pulmonary artery. Br. J. Pharmacol. 119, 149–157.

O'Rourke, S.T., Vanhoutte, P.M., 1987. Subtype of muscarinic receptors on adrenergic nerves and vascular smooth muscle of the canine saphenous vein. J. Pharmacol. Exp. Ther. 241, 64–76.

Obi, T., Kabeyama, A., Nishio, A., 1995. Characterization of muscarinic receptor subtype mediating contraction and relaxation in equine coronary artery in vitro. J. Vet. Pharmacol. Ther. 17, 226–231.

Pfaffendorf, M., Van Zwieten, P.A., 1993. Mediation by the same muscarinic receptor subtype of phasic and tonic contractile activities in the rat isolated portal vein. Br. J. Pharmacol. 108, 132–138.

Silveira, C.L.P., Eldefrawi, A.T., Eldefrawi, M.E., 1990. Putative muscarinic M_2 receptors of rat heart have high affinity for organophosphorus anticholinesterases. Toxicol. Appl. Pharmacol. 103, 474–481.

Sim, M.K., Singh, M., 1987. Decreased responsivness of the aortae of

hypertensive rats to acetylcholine, histamine and noradrenaline. Br. J. Pharmacol. 90, 147–150.

Solberg, Y., Belking, M., 1997. The role of excitotoxicity in organophosphorous nerve agents central poisoning. Trends Pharmacol. Sci. 18, 183–185.

Stewart, W.C., McKay, D.H., 1961. Some respiratory and cardiovascular effects of gradual sarin poisoning in the rat. Can. J. Physiol. Pharmacol. 39, 1001–1011.

Su, N., Narayanan, N., 1993. Age related alteration in cholinergic but not α-adrenergic response of rat coronary artery vasculature. Cardiovasc. Res. 27, 284–290.

Trendelenburg, U., 1972. Classification of sympathomimetic amines. Catecholamines. In: Blaschko, H., Muscholl, E. (Eds.), Handb. Exp. Pharmacol. vol. 33 Springer, Berlin, pp. 336–362.

Ward, T.R., Ferris, D.J., Tilson, H.A., Mundy, W.R., 1993. Correlation of the anticholinesterase activity of a series of organophosphates with their ability to compete with agonist binding to muscarinic receptors. Toxicol. Appl. Pharmacol. 122, 300–307.

Watson, N., Eglen, R.M., 1994. Muscarinic M_3 receptors mediate contraction in rabbit endothelium-denuded aorta in vitro. J. Auton. Pharmacol. 14, 283–293.