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Paraoxon-induced changes in the level of cerebral acetylcholinesterase activity in diabetic mice

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The central effects of a number of substances are known to be modified by the diabetic state (Wisniewski & Buczko 1967) or the administration of insulin (Leblanc 1960; Jasmin & Bonin 1960). It was previously reported that the administration of paraoxon resulted in significantly less accumulation of acetylcholine in the brains of diabetic than normal rats (Matin and Kar 1972). The organophosphorous compounds inhibit cholinesterase activity which is found in the central nervous system and other parts of body (Holmstedt 1959). The effect of these compounds on the level of cholinesterase in diabetic or insulin-treated animals has not been determined. We now report the effect of paraoxon on the level of cerebral cholinesterase activity in diabetic mice.

Methods

Adult male albino mice, 20–25 g, were fasted for 18 h before use since preliminary experiments indicated that this resulted in more uniform results. The animals were made diabetic by the intraperitoneal injection of alloxan (120 mg kg^{-1}) twice with a 48 h interval. All the diabetic animals used had a blood glucose level above 200 mg %. Control animals with the blood glucose level of less than 120 mg % were taken as normal. Paraoxon (0·05 mg kg^{-1}) was injected intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) according to the method of Haley & McCormick (1957) 24 h after alloxan treatment; the volume did not exceed 0·04 ml for one i.c.v. treatment. The animals were also treated with insulin (0·5 μ100 g^{-1}, i.p.) alone or with paraoxon as given in Table 1. Controls had 0·9% NaCl (i.p. or i.c.v.). The animals were killed 1 h after treatment with paraoxon. Blood glucose level was determined by the method of Nelson (1944). The acetylcholinesterase activity of the cerebral hemispheres was determined spectrophotometrically according to the method of Ellman et al (1961). The data were analysed using Student's *t*-test.

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Results and discussion

Changes in the level of cerebral acetylcholinesterase activity after the administration of paraoxon or insulin in normal and diabetic mice are given in Table 1. The i.c.v. administration of paraoxon resulted in almost equal inhibition of cholinesterase in normal and diabetic mice.

The results indicate that the level of cerebral cholinesterase activity in diabetic animals was not significantly different from the corresponding values in normal animals. Further administration of insulin had no effect on the level of cholinesterase in normal or diabetic animals (Table 1). Thus the diabetic state itself does not seem to influence the level of cholinesterase activity in the brain. However, paraoxon, given i.p., produced significantly less inhibition of cerebral cholinesterase activity in diabetic animals than in normal animals. It was previously reported that the pharmacological effects of a number of drugs (e.g. phenothiazines, analgesics) were reduced in the diabetic state (Wisniewski & Buczko 1967) and enhanced by the administration of insulin (Wisniewski 1964; Wisniewski & Danysz 1966). The mechanism of these effects is not well understood.

Insulin also increases the permeability of various biological membranes to carbohydrates (Levine et al 1949, 1950) and certain amino acids (Manchester & Wool 1963). Insulin

Table 1. Effect of paraoxon on the level of cerebral acetylcholinesterase activity in normal and diabetic mice. The animals were treated with paraoxon (0·05 mg kg^{-1}, i.p. or i.c.v.) or insulin (0·5 u/100 g.i.p.). The animals were killed 1 h after the administration of paraoxon. Each group consisted of 6 animals.

	Cerebral acetylcholinesterase activity[a]					
	I 0·9% Nacl		II Paraoxon		III Paraoxon (i.p.) +	IV
	i.p.	i.c.v.	i.p.	i.c.v.	Insulin (i.p.)	Insulin (i.p.)
Normal	7·84 ± 0·26	7·91 ± 0·42	4·17 ± 0·12[b,d]	2·37 ± 0·11[b]	3·70 ± 0·12[b]	8·31 ± 0·24
Diabetic	8·18 ± 0·18	8·06 ± 0·38	6·21 ± 0·21[c]	2·52 ± 0·18[b]	3·98 ± 0·15[b]	7·95 ± 0·32

a. Acetylcholinesterase activity expressed as moles of substrate (acetylthiocholine) hydrolysed min^{-1} g^{-1} × 10^{-6}.
b. Significantly different from the corresponding values in group I. $P < 0·01$.
c. Significantly different from the corresponding values in group II. $P < 0·05$.
d. Significantly different from the corresponding values in diabetic animals. $P < 0·01$.

may thus increase the effects of various drugs by enhancing their absorption or passage through the body membranes (Wisniewski & Malyszko 1966; Leblanc 1960). It is possible that in alloxan-induced diabetes, where there is a deficiency of insulin in the body, the permeability of various biological membranes to paraoxon might be impaired. This would reduce access of paraoxon to cerebral cholinesterase and hence may reduce the degree of inhibition. Support for this suggestion is gained from the finding that the i.c.v. administration of paraoxon resulted in an almost equal degree of cholinesterase inhibition in diabetic and normal animals. Additional support is also obtained from our results that the administration of insulin along with paraoxon given intraperitoneally, enhanced its cholinesterase inhibiting activity to a level close to that seen in normal animals (Table 1). Since the inhibition of cholinesterase is directly related to the toxicity of organophosphorous compounds (Holmstedt et al 1967; Holmstedt 1959), it is possible that the diabetic state may in some way influence or modify the toxicity of organophosphorous compounds.

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Histamine receptor agonists and antagonists on granulocyte adherence

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Over recent years it has become apparent that histamine, apart from having a role as a mediator of the inflammatory changes in blood vessels, may have an equally important role as a regulator of leucocyte function. For example it has been shown to inhibit antigen-induced release of histamine from basophils (Lichtenstein & Gillespie 1973) and to inhibit zymosan-induced lysosomal enzyme release from cytochalasin B pretreated leucocytes (Busse & Sosman 1976). Others have demonstrated that directional movement towards endotoxin-activated serum, either or neutrophils or of purified eosinophils, can be inhibited by histamine (Melmon et al 1972; Wadee et al 1980; Radermecker & Maldague 1981). The evidence suggests that inhibition of secretion and inhibition of chemotaxis by histamine are both H_2-receptor mediated phenomena (Lichtenstein & Gillespie 1973; Radermecker & Maldague 1981), and are accompanied by a rise in intracellular cyclic 3′, 5′adenosine monophosphate (cAMP) levels (Lichstenstein & Gillespie 1973; Wadee et al 1980). The present study has looked at the effect of histamine on another cell function, namely in vitro granulocyte adherence, and investigates the type of receptor involved. The effect of clonidine which has been shown to stimulate H_2-receptors in guinea-pig atria (McCulloch et al 1980) and in the central nervous system (Karppanen et al 1976) was also studied.

Methods

Measurement of granulocyte adherence. Adherence was determined by a modification of the method of Kvarstein (1969) using freshly donated human blood. 20 ml of blood were taken by venepuncture from a vein in the antecubital fossa and placed in a plastic universal tube containing 1 ml of preservative-free heparin (1000 u ml^{-1}). Following incubation with drugs at 37 °C, 0·5 ml aliquots of blood were pipetted into pre-warmed columns of glass beads. The blood was blown into the beads so that the top of the blood was level with the top of the beads, and the column was placed vertically in a rack in an incubator. The top of the column was then connected to a constant infusion pump (Palmer) via a length of polythene tubing. The pump was set up with ten 1 ml syringes in parallel so that ten samples could be perfused at the same time. The speed was adjusted to move the plungers at a rate of 1 inch min^{-1} (equivalent to 0·45 ml min^{-1}). Further columns were set up at 1 min intervals as the drug incubation period ended. Blood samples were collected from the bottom of each column into plastic blood tubes. These were stored at 5 °C until the haematology was performed.

Preparation of columns. Short (150 mm) glass Pasteur pipettes (Bilbate) and glass ballotini (Grade No. 8) with an approximate size range of 440—530 μm (Jencons) were