Action of clonidine on the mast cells of rats

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Clonidine is known to possess peripheral α-adrenoceptor stimulating properties in addition to its potent central hypotensive effect (Nickerson & Ruedy 1975). Its action resembles that of histamine in that (a) the positive inotropic effect in isolated hearts has a similar mode of action to that of histamine (Csongrady & Kobinger 1974) and (b) it stimulates gastric acid secretion in the anaesthetized rat, which is thought to be mediated through the release of endogenous histamine (Jennewein 1977; Parsons 1978). Furthermore, clinically it has been observed that chronic treatment with clonidine produces side effects like allergic manifestations, including rashes, pruritus and angio-neurotic oedema (Nickerson & Ruedy 1975). Since α-adrenoceptor stimulants like phenylephrine, noradrenaline and imidazole are known to degranulate and release histamine from the mast cells of rat (Kato & Goeszy 1966; De Oliveira & Rothschild 1968; Mannaioni et al 1968; Moroni et al 1977; Taylor et al 1974) and histamine plays a significant role in mediating the allergic manifestations, we have investigated the effect of clonidine on the mast cells of rats.

Charles Foster rats (150–200 g) of either sex were used. Peritoneal mast cells were harvested according to Garland & Mongar (1974). The peritoneal washings from six rats were pooled and centrifuged at 2000 rev min^{-1}. The mast cells were suspended in 6 ml of Tyrode solution. Various concentrations of clonidine (10–80 μg ml^{-1}) were added to test tubes containing porting (0·5 ml in duplicate) of cell suspension. These were incubated at 37 °C in a metabolic shaker for 10 min, after which cell suspensions were centrifuged and the supernatant decanted and kept on ice for histamine assay. The mast cells were resuspended in 2 ml of Tyrode solution and boiled for 10 min to obtain the residual histamine. Released and residual histamine were assayed on guinea-pig ileum suspended in Tyrode solution at 37 °C containing atropine (2×10^{-7} g ml^{-1}) and methysergide (2×10^{-7} g ml^{-1}). The released histamine (supernatant histamine) was expressed as a percentage of the total histamine present in the cells (released plus residual) after correcting for the spontaneous release in Tyrode solution alone.

The results show that rat peritoneal mast cells, when incubated with different concentrations of clonidine, release histamine in a dose dependent manner (Fig. 1).

The effects of various histamine liberators on the degranulation of mast cells has been classified by Stanworth (1973) into selective and non-selective agents. Selective agents act through the dynamic expulsion of granules without causing any damage to the cell wall, while non-selective agents cause drastic general effects upon the cell and cytoplasmic membranes, which ultimately degranulate the mast cells by deterioration of the cell wall. To determine the nature of degranulation caused by clonidine, six rats (150–200 g) were treated with (0·05, 0·1 and 0·2 mg kg^{-1}) daily, by intraperitoneal administration for 3 days. Two other groups of six rats each were treated with compound 48/80 (0·1, 0·3 and 0·5 mg kg^{-1}) and octylamine (0·5, 1·0 and 2·0 mg kg^{-1}) respectively in a similar manner. At the end of the treatment, loose areolar tissue from two different sites of the intrascapular region was removed and spread on a microscope slide, fixed with 5% formalin in 50% methanol and stained with 1% Toluidine blue containing 0·1% acetic acid. The mean percentage of degranulated mast cells was determined by counting 500 cells from each subcutaneous spread. Significance was calculated by applying Student's *t*-test.

The chronic administrations of different doses of clonidine, compound 48/80 and octylamine in rats caused significantly more degranulation of mast cells compared with the control group in a dose dependent manner (Fig. 2). The microscopic studies of the subcutaneous tissue spreads from the animals treated with clonidine and compound 48/80 revealed the degranulations, indicated by the presence of vacuoles within the mast cells. The density and homogenity of the granules were reduced. Granules were seen discharged from the cells, while cell walls were found intact. In contrast, octylamine produced more drastic general effects upon

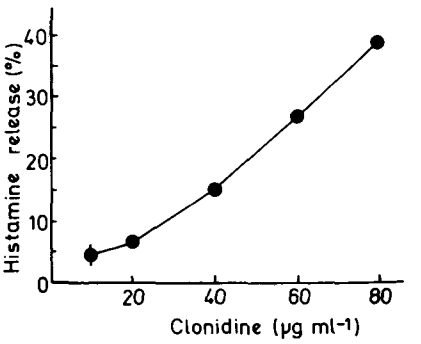

FIG. 1. Histamine release from rat peritoneal mast cells induced by various concentrations of clonidine. Each point represents the mean ● s.e. of six experiments.

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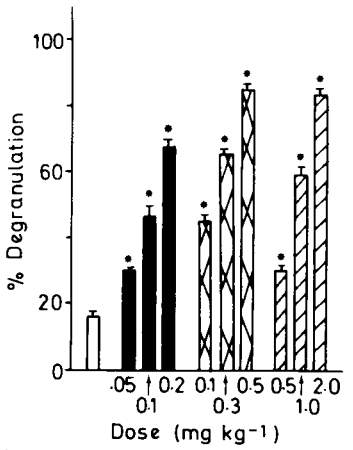

FIG. 2. Histogram representing the mean percentage degranulation of mast cells after the treatment with clonidine (0·05, 0·1 and 0·2 mg kg^{-1} i.p.), compound 48/80 (0·1, 0·3 and 0·5 mg kg^{-1} i.p.) and octylamine (0·5, 1·0 and 2·0 mg kg^{-1} i.p.) daily for 3 days. Vertical bars show s.e.m. (n = 12). Value of significance shown is * $P < 0.001$ with Student's t-test.

the cell walls, causing the degranulation by disruption of the cell walls. Compared with the foregoing findings, mast cells from the control group exhibited the presence of granules as homogenous, dense-staining cytoplasmic inclusions within the intact cell wall. It is therefore conceivable that clonidine degranulates mast cells in a manner similar to a selective liberator like compound 48/80 and unlike octylamine which is a non-selective histamine-releasing agent.

Thus, our results show that clonidine can affect rat mast cells by evoking a selective exocytosis. This, in turn, may lead to the gastric acid secretion and mediation of allergic manifestations.

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Inhibition by caffeine of calcium uptake by brain microsomal vesicles*

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Subcellular vesicles obtained from brain homogenates through various procedures, take up calcium by an ATP dependent oxalate- or phosphate-enhanced process mediated by a membrane-bound ATPase (Trotta & de Meis 1975, 1978; Kendrick et al 1977; Blitz et al 1977). This calcium uptake process is probably one of the mechanisms that regulate the concentration of free calcium ions in the cytosol of nerve cells, since increasing evidence indicates that most of the internal calcium of nerve is sequestered in intracellular compartments by a process requiring ATP (Hodgkin & Keynes 1957; Blaustein & Hodgkin 1969; Baker et al 1971; Baker 1976; Scarpa et al 1977).

It is well known that caffeine is a potent central nervous system stimulant, however, the mechanism by which it exerts its stimulating effect remains unclear (Ritchie 1975). It is not known whether caffeine-induced variations of intracellular cAMP concentration could explain the drug's effects on brain function (Butcher & Sutherland 1962; Ritchie 1975). It is known that both calcium and cAMP are ubiquitous regulators of cellular functions, and may interract in the modulation of various metabolic functions (Rasmussen 1970). It is also known that the concentration of calcium ions in the cytosol of nerve cells regulates excitability, nervous conduction and transmitter release (Grundfest et al 1954; Katz & Miledi 1967, 1970; Holz 1975). Consequently, it is possible that a caffeine-induced modification of calcium uptake by brain microsomal vesicles, which would result in a modification in the concentration of calcium ions in the cytosol of brain cells, could provide an additional biochemical basis for the explanation of the effects of caffeine in these

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