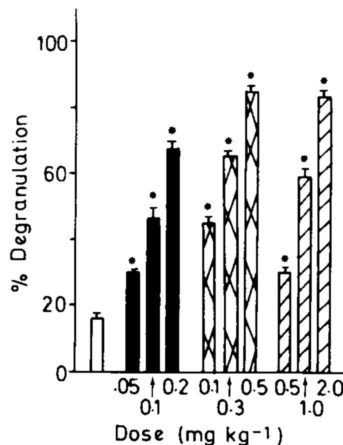

FIG. 2. Histogram representing the mean percentage degranulation of mast cells after the treatment with clonidine (0·05, 0·1 and 0·2 mg kg^{-1} i.p.), compound 48/80 (0·1, 0·3 and 0·5 mg kg^{-1} i.p.) and octylamine (0·5, 1·0 and 2·0 mg kg^{-1} i.p.) daily for 3 days. Vertical bars show s.e.m. (n = 12). Value of significance shown is * $P < 0{\cdot}001$ 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.

The authors wish to thank Mr D. R. Hanamsheth for skilful technical assistance.

February 25, 1980

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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 cystosol 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

* This investigation was supported by Conselho Nacional de Desenvolvimento Cientifico e Technológico (CNPq).

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cells. The experiments described in this report were designed to test this hypothesis.

Microsomal fraction was prepared from rabbit brain as previously described (Trotta & de Meis 1975, 1978).

Calcium uptake was measured using $^{45}CaCl_2$ as previously described (Trotta & de Meis 1975). The assay medium contained Tris-maleate buffer pH 7·0 40, KCl 50, ouabain 0·1, $MgCl_2$ 5, ATP 2·5, $CaCl_2$ 70, potassium phosphate buffer pH 7·0 70 mM and 0·4 mg ml^{-1} of microsomal protein. The reaction was started by the addition of the microsomal fraction and carried out at 30 °C; it was stopped by Millipore filtration (type HA 0·45 μm pore size). The amount of calcium taken up by the vesicles was calculated from the difference between the radioactivity of the filtrate and the initial radioactivity of the assay medium.

The time course of calcium uptake in the absence or presence of caffeine is shown in Fig. 1. Caffeine inhibits the rate of calcium uptake and the steady-state level of calcium accumulation in the vesicles. The percent inhibition of calcium uptake by 5, 10 and 15 mM caffeine was 16, 33 and 50% respectively.

The effects of caffeine on calcium ions has been studied in detail in skeletal muscle. It was shown that low concentrations of caffeine strengthen muscle contraction and higher concentrations produce contracture (Bianchi 1968, 1975). It was also shown that caffeine impairs calcium uptake and produces calcium release by isolated sarcoplasmic reticulum, which accounts for the effects of caffeine in whole muscle (Johnson & Inesi 1969; Fairhurst & Hasselbach 1970; Batra 1974; Ritchie 1975). The degree of inhibition of calcium uptake in the sarcoplasmic reticulum by caffeine is similar to that described herein for brain microsomal vesicles in equivalent caffeine concentrations. The observation that caffeine also inhibits calcium uptake by brain microsomal vesicles supports the idea that it could have a parallel mode of action in both brain and muscle.

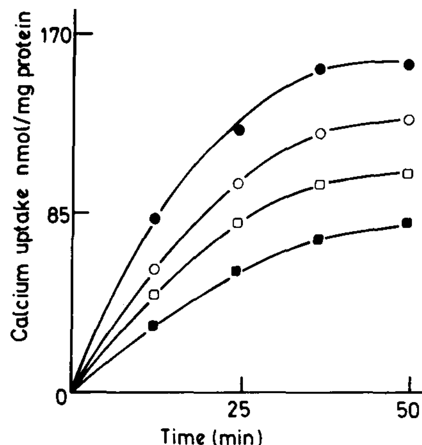

FIG. 1. Time course of calcium uptake by brain microsomal vesicles in the absence or presence of caffeine. Calcium uptake was assayed in the absence of caffeine (●) or in the presence of 5 mM caffeine (○), 10 mM caffeine (□), or 15 mM caffeine (■).

Table 1. Reversal of caffeine-induced calcium uptake inhibition by amethocaine. Brain microsomal vesicles were allowed to take up calcium for 45 min in the standard assay medium described in test, in the absence or presence of caffeine or amethocaine.

Additions to assay medium	Calcium uptake (nmol mg^{-1} protein)
none	151·3
10 mM Caffeine	98·4
3 mM Amethocaine	156·2
10 mM Caffeine + 3 mM amethocaine	154·7

It has been shown that local anaesthetics counteract the effects of caffeine in whole muscle as well as in fragmented sarcoplasmic reticulum. Table 1 shows that amethocaine (tetracaine) also counteracts the inhibitory effect of caffeine on calcium uptake by brain microsomal vesicles. In unpublished work from this laboratory the effects of local anaesthetics on the calcium pump of brain were studied in detail. In the experiment of Table 1 we have used a concentration of tetracaine that does not significantly modify calcium uptake. The data are reported solely to provide additional information on the similarities between the effects of caffeine on the calcium pump of both brain and muscle.

Although the nature and cellular localization of calcium sequestering vesicles of brain has not been determined, recent findings suggest that these vesicles are probably located on the synaptic region (Kendrick et al 1977; Blitz et al 1977). It is known that transmitter release is regulated by the concentration of calcium ions inside nerve terminals (Katz & Miledi 1967, 1970). Consequently, a caffeine-induced inhibition of calcium uptake by brain subcellular vesicles, which would increase calcium ions concentration in nerve terminals, could produce a facilitation of transmitter release that could result in a stimulatory effect of central nervous system. These findings suggest that the pharmacological effects of caffeine in both brain and muscle could be at least in part explained by a common mechanism.

March 10, 1980

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Kinetics of extraneuronal uptake of isoprenaline in trachealis smooth muscle cells: comparison of rat and guinea-pig

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Bryan & O'Donnell (1980a) have used fluorescence microphotometry to determine the relative affinities of noradrenaline, adrenaline and isoprenaline for extraneuronal uptake specifically in smooth muscle cells. The cells examined were in the trachealis muscle of guinea-pig trachea. In the present study the kinetics of extraneuronal uptake of isoprenaline in the same cell type but in a different species, rat, has been examined.

Adult, male rats (325–425 g), pretreated with 50 mg kg^{-1} 6-hydroxydopamine intravenously 24 h before the experiment, were killed by a blow on the head. The trachea was removed and cut into six to eight rings which were randomly assigned to the treatments in each experiment. The tracheal rings were washed and incubated in (±)-isoprenaline at 37 °C as described by Bryan & O'Donnell (1980a). Catechol-*O*-methyl transferase was inhibited by inclusion of 100 μM U-0521 in the Krebs solution (Bryan & O'Donnell 1979). After washing at 0–1 °C, the tissues were prepared for fluorescence histochemistry by the Falck-Hillarp technique (Falck 1962) as described by Anning et al (1979). Fluorescence intensities (in arbitrary fluorescence units, F) in areas of trachealis smooth muscle 2·5 μm square were measured and the intensity values were corrected for background fluorescence as described by Bryan & O'Donnell (1980b). All drugs and solutions were prepared as described by Bryan & O'Donnell (1980a).

In a preliminary experiment with 800 μM isoprenaline, uptake of isoprenaline by the trachealis smooth muscle cells was measured at incubation times of 2, 5, 10, 15, 20, 30 and 60 min. Uptake occurred at constant rate up to 5 min, then gradually decreased in rate at the longer incubation times. Hence, an incubation time of 4 min in isoprenaline was used in the subsequent initial rate study.

Initial rates of uptake of isoprenaline into trachealis smooth muscle cells were determined for amine concentrations of 50, 100, 200, 400 and 800 μM. Plots of mean initial rate of uptake (v) from 5 rats against isoprenaline concentration (s) (Fig. 1A) and of s/v against s (Fig. 1B) indicated that the uptake obeyed Michaelis-Menten saturation kinetics as was found for the same cells in guinea-pig (Bryan & O'Donnell 1980a). For each animal, K_m and V_{max} values were determined by weighted regression analysis according to the method of Wilkinson (1961). For rat trachealis smooth muscle, $K_m = 484 \pm 32{\cdot}8$ μM and $V_{max} = 441 \pm 16{\cdot}4$ F min^{-1} (mean ± s.e. from 5 rats), compared with $K_m = 273 \pm 12{\cdot}1$ μM and $V_{max} = 257 \pm 6{\cdot}6$ F min^{-1} in guinea-pig (Bryan & O'Donnell 1980a). The K_m and V_{max} values were both significantly greater in rat than in guinea-pig (K_m: $t = 6{\cdot}04$, d.f. 8, $P < 0{\cdot}001$; V_{max}: $t = 10{\cdot}41$, d.f. 8, $P < 0{\cdot}001$; Student's *t*-test).

These results indicate that the extraneuronal uptake mechanism for isoprenaline in trachealis smooth muscle cells in rat has a slightly lower affinity but also a slightly higher capacity than was found in guinea-pig cells. Thus, for those experimentally used substrate concentrations less than 400 μM, the rates of uptake of isoprenaline in the two species did not differ significantly, but at higher concentrations the rate of uptake was significantly greater in rat than in guinea-pig. It could be predicted from the kinetic parameters that at low isoprenaline concentrations (less than 20 μM) the reverse order would apply. These observations suggest that the small differences between the kinetic parameters of extraneuronal uptake in rat and guinea-pig trachea are unlikely to be of pharmacological signifi-

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