Preliminary Report on the Incorporation of Guanethidine and Reserpine into Rat Peritoneal Mast Cells in vitro

Guanethidine and reserpine have been extensively used in the study of monoamines in the central nervous system and in several nerve ending models[1-4]. The mechanisms by which these drugs exert their effects on the amine stores are still in many respects unknown. Evidence suggesting an uptake of guanethidine by an energy-requiring process has been presented[5-7]. Guanethidine is known to accumulate in human platelets by an active mechanism[8] and the uptake by heart slices of this drug takes place by two different mechanisms, one of which is active[9]. Reserpine has been demonstrated to interfere with guanethidine uptake and binding in rat heart in vivo[10]. Reserpine is also incorporated into rabbit blood platelets and becomes bound to the platelet granular membrane[11,12]. It has been shown by fractional studies that the location of reserpine in the mouse heart is mainly in the heavy fraction[13], and failure to find incorporation of reserpine into specific amine storing organelles has been reported[14]. Since reserpine is known to affect the endogenous content of 5-HT and depresses the uptake of this amine into mast cells, and both guanethidine and reserpine affect the uptake of 5-HT into isolated mast cell granules[15-17], a study on the incorporation of these drugs into mast cells seemed motivated.

Adult Sprague-Dawley rats of both sexes were used. Peritoneal cells were washed out from the abdominal and pleural cavities with 10 ml of an ice-chilled modified[18] Krebs-Ringer-glucose solution (KRG). The cells were spun down at 350 $g \times 10$ min, resuspended in fresh salt solution and spun down a density gradient made up of 30% Ficoll (Pharmacia) in saline for 15 min at 350 g. The 30% layer containing some 60–80% of the total mast cell count and with a degree of purity of the mast cells ranging from 70 to 95% was collected. The cells were washed twice with fresh KRG, counted in a Buerker chamber and used in incubation experiments.

The leucocytes remained above the 30% Ficoll layer and were also collected and processed identically to the mast cell fraction. All the experiments except those concerning the temperature dependence of incorporation were carried out in a water bath under agitation. The drug concentrations used were $5 \times 10^{-6} M$ for reserpine (Sigma Chemical Co.) and $5 \times 10^{-5} M$ for guanethidine (Ciba AG), referring to the bases. After incubation the cells were spun down and washed three times before assay. The mast cells were degranulated by freezing and thawing 3 times in 0.3 M sucrose pH 6.9. Nuclear and granular fractions were collected by differential centrifugation at 350 $g \times$ 10 min and 2700 $g \times$ 30 min, respectively. Both fractions and final supernatants were assayed. 5-HT was assayed spectrofotofluorometrically using the method of Bogdanski as described by Weissbach[19]. Reserpine was assayed fluorometrically after chloroform extraction according to Jakovlevic et al.[20]. Guanethidine was assayed spectrofotofluorometrically according to a semimicro modification of the method given by Schanker et al.[9]. The same samples were used for the determination of 5-HT and both drugs.

The cellular distribution studies showed that within the peritoneal cells, reserpine and guanethidine are taken up into mast cells as well as leucocytes. The amount of guanethidine incorporated was 98.3 ng/10^6 mast cells and 32.5 ng/10^6 leucocytes in 1 h at 37°C (17 and 4 experiments, respectively, incubated and determined in triplicate). The corresponding figures for reserpine were 227 ng/10^6 mast cells and 54.8 ng/10^6 leucocytes (21 experiments incubated and determined in triplicate). One may speculate as to whether this specificity of drug binding to mast cells is in some way related to other specific characteristics of mast cells, e.g. their capacity to take up and store biogenic amines, or whether it merly reflects the greater intracellular volume of mast cells compared with leucocytes.

Table I. Distribution of 5-HT, guanethidine and reserpine in subcellular fractions of isolated rat peritoneal mast cells

Fraction	Guanethidine (%)	5-HT (%)	Reserpine (%)	5-HT (%)
350 xg	64	66	57	58
2700 xg	36	34	43	42

Distribution expressed as a percentage of the total spun down (nuclear and granular fractions). Degranulation and differential centrifugation as described in text. Means of 5 guanethidine and 5 reserpine experiments. Figures rounded off.

Table II. Incorporation of guanethidine and reserpine into isolated rat peritoneal mast cells

Drug added	Guanethidine ng/10^6 mast cells	Reserpine ng/10^6 mast cells
Control	95.7 ± 14.7	302.5 ± 6.7
Both drugs	93.0 ± 12.0	301.7 ± 7.3

Incubation in KRG at 37 °C for 60 min. Drug concentrations: guanethidine $5 \times 10^{-5} M$, and reserpine $5 \times 10^{-6} M$. Means and S.E. of 2 experiments incubated and determined in triplicate. Control: either guanethidine or reserpine added to the medium. Both drugs: Guanethidine and reserpine added to the medium simultaneously.

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The incorporation of reserpine differed markedly from that of guanethidine in that it was completely independent of temperature, the uptake being about the same at 0.23 and 37°C. The incorporation of guanethidine was temperature dependent, being 10% at 0°C and 35% at 23°C of the uptake at 37°C. The incorporation of neither drug was inhibited by NaCN at 10^{-5} *M* of FCCP at 3×10^{-6} *M*. Incubation of mast cells with 5-HT added 30 min before the addition of reserpine did not affect the uptake of reserpine even at a 5-HT concentration of 2.5×10^{-5} *M*. The incorporation of guanethidine was reduced to about 50% of the control level by 5-HT at 6×10^{-6} *M* added 15 min before the addition of guanethidine.

The intracellular location of both drugs seems to be mainly granular, judging from the assay of nuclear and granular fractions collected as described above after incubation with drugs. The percentual distribution of 5-HT and guanethidine and 5-HT and reserpine was almost identical (Table I). The subcellular location of 5-HT and histamine is mainly granular[16,21,22]. Taking 5-HT as a granular marker, this leads to the conclusion that reserpine and guanethidine are almost exclusively located in the amine storing granules in mast cells.

Both drugs seem to become incorporated into mast cells independently of each other since reserpine at 5×10^{-6} *M* did not affect the uptake of guanethidine. Neither did guanethidine at 5×10^{-5} *M* affect the uptake of reserpine (Table II).

These preliminary results indicating incorporation of both drugs into mast cells, where the drugs become bound to the amine storing gramules, are in close correspondence with earlier observations[15-17] showing that both drugs tested interfere with 5-HT kinetics in mast cells. Experiments designed to reveal the exact mechanisms underlying these effects and to explain the difference in action of reserpine and guanethidine on 5-HT kinetics in mast cells and simple neuronal models are in progress.

Zusammenfassung. Isolierte peritoneale Mastzellen der Ratte wurden in KRG-Puffer mit Reserpin und Guanethidin inkubiert. Beide Substanzen scheinen unabhängig voneinander aufgenommen zu werden, da die gleichzeitige Inkubation mit Reserpin und Guanethidin die Aufnahme im Vergleich mit den Kontrollen nicht herabsetzt.

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Hyperthermic Effect of Disodium Edetate Injected into the Lateral Cerebral Ventricle of the Unanesthetized Cat

FELDBERG et al.[1] reported that perfusion of physiological NaCl solution through the cerebral ventricular system of unanesthetized cats resulted in the rapid development of high fevers, whereas body temperature was not altered if the solution also contained a physiological concentration of calcium ion. A later report[2] extended these observations to the unanesthetized rabbit and also demonstrated that increasing calcium ion concentrations above those normally present in cerebrospinal fluid (CSF) caused hypothermia in some animals and antagonized the pyrogenic effect of leukocytic pyrogen. Similar effects have also been produced in unanesthetized cats by perfusion in the posterior hypothalamus[3]. No change in temperature was produced, however, provided the relative concentrations of sodium and calcium ions in the perfusing fluid were kept the same as those in extracellular fluid. The authors suggested that the balance between sodium and calcium ions in the hypothalamus may be responsible for determining the set point of the thermoregulatory thermostat[1-3] and that pyrogens may act by altering this balance[2]. The purpose of the present experiments was to determine the effect on body temperature of calcium ion binding in CSF by the chelating agent disodium edetate (Na_2EDTA).

Methods and materials. Cats, weighing between 2.4 and 5.0 kg, were prepared with lateral cerebral ventricular cannulas, jugular venous catheters and retroperitoneal thermocouples as in previous experiments[4]. Body temperature was recorded automatically on a multipoint recorder at intervals of 3 min during the 1st h after each test injection and at least every 15 min thereafter until recovery. The average of temperature readings 0, 15 and 30 min before ventricular injection was used as the baseline from which changes were measured. Environmental temperature was maintained at 75 ± 2°F. Ventricular injections (all 0.10 ml in volume) were made at the same time of day in each cat, usually at daily intervals. Antipyretics were administered i.v., 30 min before ventricular injections of Na_2EDTA, and were flushed in with 1.0 ml of saline solution. Cannulas and catheters were also flushed 3–4 h before tests. A Harvard syringe pump was used for infusions into the ventricular cannulas.

Commercial, nonpyrogenic saline solution was used for all solutions, control injections and flushes. All containers, syringes and needles were either of the commercial, nonpyrogenic, disposable type or were sterilized in dry heat at over 200°C for at least 2 h. Stock solutions of $Na_2EDTA \cdot 2H_2O$ and of calcium disodium edetate ($CaNa_2EDTA$) were stored at 4°C until needed. Fresh solutions of sodium salicylate (100 mg/ml) and acetaminophen (10 mg/ml) were prepared for each injection.

Results. Dose-related hyperthermic responses were produced by intraventricular injections of Na_2EDTA. Figure 1 shows responses to various doses in one of the cats. A 200 µg dose was effective in all cats. Tremor of the ears usually developed within 30 sec, followed by a fine

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