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SECRETION FROM RAT BASOPHILIC LEUKAEMIA CELLS INDUCED BY CALCIUM IONOPHORES

EFFECT OF pH AND METABOLIC INHIBITION

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Previous experiments on the functional properties of rat basophilic leukaemia cells showed a major anomaly when compared to normal mast cells: though IgE-mediated secretion was dependent on external Ca^{2+} with both types of cells, substantial non-cytotoxic release with ionophore A23187 could be demonstrated with the normal cells but not with the tumour cells. We now show that when the pH of the incubation medium is increased to 8 it is possible to obtain excellent Ca-dependent, non-cytotoxic secretion from tumour basophils with the ionophores A23187 and ionomycin. These results provide further evidence that secretion from the tumour cells occurs via a mechanism similar to that used by normal mast cells and basophils. Experiments with metabolically inhibited tumour cells suggest that their unusual sensitivity to the cytotoxic effects of Ca^{2+} ionophores may be related to their ability to sequester intracellular calcium. Changes in the conditions of cell culture appeared to produce substantial and at least partially reversible changes in responsiveness to IgE-mediated triggering and ionophores.

Introduction

Rat basophilic leukaemia cells, which can be grown in vitro and in large numbers, provide a unique opportunity for exploring the molecular details of IgE-mediated cell activation at a level that would be difficult with normal cells. Secretion from tumour basophils resembles that from normal mast cells and basophils in many respects [1–3]. However, certain differences do exist and so the degreee to which insights into the function of the tumour cells are applicable to their normal counterparts is not always easy to assess [3].

It is now generally accepted that exocytosis from most secretory cells occurs as a result of an increase in free ionized calcium in the cytoplasm of these cells [4,5]. One of the important pieces of evidence in support of this hypothesis was the demonstration that the normal IgE-mediated activation of mast cells could be mimicked by the direct introduction of Ca^{2+} into the cytoplasm using the divalent cation ionophore A23187 [6]. Since then, A23187 induced secretion has been demonstrated in numerous other systems from diverse species and the ionophore has become an important diagnostic tool in the study of calcium-dependent cell activation.

A particularly striking exception was the finding that although IgE-mediated secretion from tumour basophils was clearly calcium-dependent, these cells failed to secrete in response to non-cytotoxic concentrations of A23187 [1]. The availability of a more

Abbrevations: anti-IgE, $F(ab')_2$ fragments of purified rabbit IgG anti-rat myeloma IgE; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; EGTA, ethaneglycolbis(β-aminoethyl ether)-*N,N'*-tetraacetic acid.

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actively secreting subline (2H3 [7]) of the tumour cells, as well as of another and more highly Ca^{2+}-specific ionophore, ionomycin [8,9], prompted us to re-examine the response of the tumour cells to ionophores. A preliminary account of some of these results has been given [3].

Materials and Methods

Cells

Rat basophilic leukaemia cells of the secreting subline 2H3 (Barsumian et al., unpublished results) were maintained in monolayer cultures and harvested as described previously [10]. In some experiments 2H3 cell variants which had lost their ability to secrete in response to triggering via IgE were examined. One of these which was obtained from Dr. F.-T. Liu (Scripps Clinic and Research Foundation, La Jolla, CA 92037) had been cultured in the same way except that cells were dislodged from the monolayer during passaging using EDTA alone (rather than trypsin: EDTA). Another non-secreting variant arose when 2H3 cells were grown in spinner culture as described previously [11].

Compounds

Ionophore A23187 was kindly provided by Dr. R. Hamill of Lilly Research Laboratories, Indianapolis, IN and was stored at −20°C as a 1 mM stock solution in ethanol. Ionomycin was the gift of Dr. E. Meyers of the Squibb Insitute for Medical Research, Princeton, N.J. and was stored as a 2 mM stock solution in acetone at −20°C. Antimycin A (Sigma Chemical Co., St. Louis, Missouri) was stored as a 1 mM stock solution in ethanol at −20°C. The compounds were diluted into the experimental buffered salt solution immediately before use. The highest concentrations of ethanol (1%) and acetone (0.5%) in the final incubation mixtures had no effect on release.

Release experiments

After loading with [^{3}H]serotonin and passive sensitization with rat IgE as described previously [10], cells were washed and resuspended in a buffered salt solution of the following composition: 135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5.6 mM glucose, 0.05% gelatin and 10 mM Hepes pH 8. Glucose was omitted from the medium at this stage in metabolic inhibition experiments. Where the effect of extracellular Ca^{2+} removal was to be examined, calcium was omitted and 0.1 M EGTA was added to chelate any residual calcium.

Cells (at a final concentration of $2 \cdot 10^6$/ml) were exposed to ionophores or anti-IgE ($F(ab')_2$ fragments of purified rabbit IgG anti-rat myeloma IgE [1]) for 45 min at 37°C. In experiments with antimycin A, the cells were exposed simultaneously to the releasing stimulus and the metabolic inhibitor with or without glucose. Release of [^{3}H]serotonin was determined from the amount remaining in the supernatant after sedimenting the cells and is expressed as a percentage of the total [^{3}H]serotonin content of the cell suspension prior to centrifugation. Release of lactate dehydrogenase was assayed as described previously [10].

Results

Effect of pH

Initial experiments with the more actively secreting subline (2H3) of rat basophilic leukaemia cells yielded substantial release of incorporated [^{3}H]-serotonin only at cytotoxic doses of A23187. However, in contrast to the earlier studies [1], some non-cytotoxic secretion was observed at lower concentrations of ionophore, although this was somewhat variable and was never as great as that seen with an optimal concentration of anti-IgE.

Since both A23187 and ionomycin complex calcium more readily at higher pH [8] we examined the effect of pH on the release of [^{3}H]serotonin. At pH 8 (Fig. 1) low doses of ionophore were adequate to obtain substantial release of [^{3}H]serotonin and there was a significant separation between the concentrations required for secretion and those which were cytotoxic (as assessed by the release of the cytoplasmic enzyme lactate dehydrogenase). The [^{3}H]-serotonin release induced by A23187 increases with increasing pH while spontaneous and anti-IgE induced secretion are virtually unaffected (Fig. 2). The pH dependence of ionomycin-induced release was similar to that seen with A23187 (data not shown). At pH 8 the amount of secretion observed with optimal non-cytotoxic doses of the two ionophores was similar to the optimal release obtained with IgE-mediated triggering (Fig. 1).

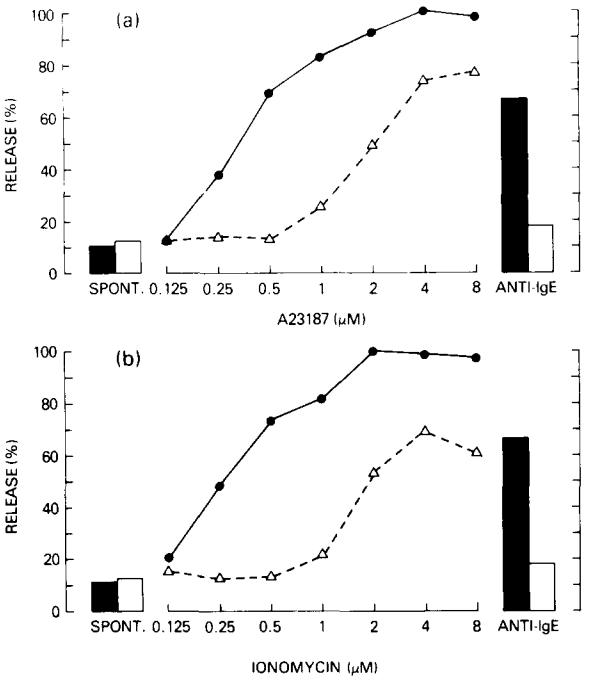

Fig. 1. Release of [^{3}H]serotonin (●——●) and lactate dehydrogenase (△– – –△) induced by ionophores from subline 2H3 of rat basophilic leukaemia cells. Spontaneous release and release induced by 2 μg/ml of F(ab')$_2$ fragments of anti-IgE are shown for comparison. Release of [^{3}H]serotonin is shown in closed columns and lactate dehydrogenase release in open columns. (a) A23187; (b) ionomycin.

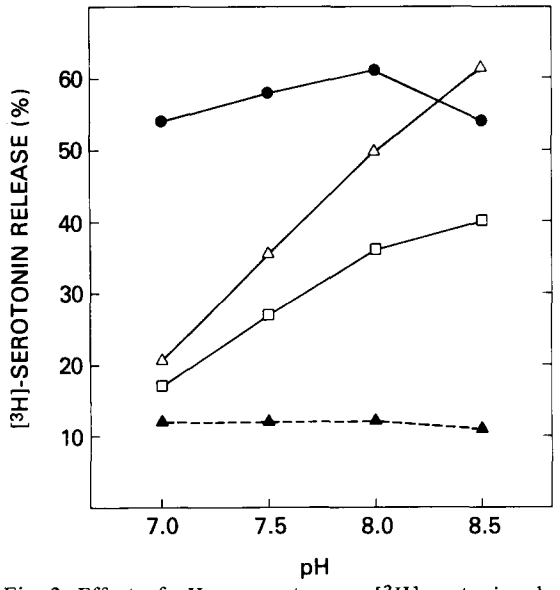

Fig. 2. Effect of pH on spontaneous [^{3}H]serotonin release (▲) and release induced by 2 μg/ml of F(ab')$_2$ fragments of anti-IgE (●), 0.5 μM A23187 (□) and 1 μM A23187 (△).

Both the cytotoxic and non-cytotoxic effects of the ionophores required extracellular Ca^{2+}, which suggests that both are due to a rise in intracellular Ca^{2+}. However the biphasic nature of the dose-response curves often seen with the two inonophores (e.g., Fig. 1b) suggests that two different processes are occurring. Thus at low ionophore concentrations limited entry of Ca^{2+} leads to exocytosis while at higher concentrations the uncontrolled entry of Ca^{2+} is cytotoxic to the cells.

Effect of metabolic inhibition

In normal rat mast cells both ionophore and IgE-mediated secretion are dependent on a source of metabolic energy. The same is true for tumour basophils, however the response of these cells to metabolic inhibitors is rather complex. The effect of glucose deprivation and of the inhibitor of oxidative phosphorylation, antimycin A, on spontaneous [^{3}H]-serotonin release and release induced by anti-IgE and the ionophore A23187 is shown in Table I. Glucose deprivation had little effect on spontaneous secretion; however, as we had found previously [2,3], antimycin A in the absence of glucose markedly potentiated the spontaneous release of [^{3}H]serotonin. This was not a cytotoxic effect since no increase in lactate dehydrogenase was observed. A similar potentiation of spontaneous secretion was seen with cyanide and dinotrophenol (unpublished observations) which affect different stages in oxidative phosphorylation. The effects of antimycin A on spontaneous and IgE-mediated secretion were not seen in the presence of glucose (Table I) which suggests that they can be overcome with ATP produced during glycolysis.

As with normal rat mast cells [6] secretion induced by ionophore was considerably reduced in the absence of glucose. However treatment with antimycin A resulted in a massive release of [^{3}H]-serotonin at least part of which was a cytotoxic effect since release of lactate dehydrogenase also occurred. When glucose was present, the cytotoxic effect of A23187 on antimycin A-treated cells was abolished and the release of [^{3}H]serotonin was considerably reduced (Table I).

The release of [^{3}H]serotonin induced by both anti-IgE and A23187 showed an absolute requirement for extracellular Ca^{2+} regardless of the state of metabolic inhibition. In contrast, the spontaneous secre-

TABLE I

EFFECT OF GLUCOSE DEPRIVATION AND OF ANTIMYCIN A ON SPONTANEOUS RELEASE OF [^{3}H]SEROTONIN AND ON RELEASE INDUCED BY F(ab')$_2$ FRAGMENTS OF ANTI-IgE AND BY A23187

	[^{3}H] Serotonin release (% ± S.D.)			
	Control		Antimycin A (5 nM)	
	No glucose	+ glucose	No glucose	+ glucose
Spontaneous	13.8 ± 0.4	11.4 ± 0.1	34.5 ± 0.4	12.5 ± 0.9
Anti-IgE (2 μg/ml)	37.6 ± 2.3	56.8 ± 2.7	35.6 ± 0.2	53.7 ± 0.9
A23187 (0.3 μM)	18.6 ± 5.1	41.3 ± 6.9	54.7 ± 0.4 *	29.4 ± 1.7

* Lactate dehydrogenase release was less than 10% except as indicated (*) where 27% release was observed.

tion induced by antimycin A in the absence of glucose was only very slightly reduced if extracellular Ca^{2+} was removed (Table II). For clarity only the results obtained with cells which were metabolically normal or completely inhibited are shown. Lactate dehydrogenase release was only observed when metabolically inhibited cells were exposed to A23187 and this cytotoxic effect of the ionophore was dependent on extracellular Ca^{2+} (Table II).

Studies with cells unresponsive to anti-IgE

Since at a slightly higher pH we had now been able to demonstrate excellent ionophore-induced non-cytotoxic release from tumour cells which could be stimulated by an IgE-mediated mechanism, it was of interest to re-examine tumour cells which have a normal capacity to bind IgE [12] but which fail to respond when reacted with anti-IgE. In previous experiments at lower pH such cells also failed to secrete at non-cytotoxic doses of ionophore [13].

Two types of pattern were seen with the unresponsive cells. In one case (Fig. 3a) the response was similar to those previously reported; no release of [^{3}H]serotonin was observed at non-cytotoxic doses of ionophore, while at higher doses the release of [^{3}H]serotonin was precisely correlated with the release of lactate dehydrogenase. The close parallelism seen with these non-secreting cells provides additional confirmation that release of lactate dehydrogenase is a sensitive measure of cytotoxic release of [^{3}H]serotonin.

It is notable that these non-responding cells had been derived from the secreting cells but had been grown in spinner cultures, rather than stationary

TABLE II

EFFECT OF EXTRACELLULAR Ca^{2+} ON SPONTANEOUS, ANTI-IgE AND A23187-INDUCED [^{3}H]SEROTONIN RELEASE FROM NORMAL AND METABOLICALLY INHIBITED CELLS

	[^{3}H] Serotonin release (% ±S.D.)			
	Metabolically normal (+ glucose 5.6 mM)		Metabolically inhibited (no glucose + antimycin A 10 nM)	
	+ Ca^{2+}	No Ca^{2+}	+ Ca^{2+}	No Ca^{2+}
Spontaneous	10.4 ± 0.3	11.2 ± 1.2	30.2 ± 0.7	26.4 ± 0.6
Anti-IgE (2 μg/ml)	38.4 ± 0.5	11.0 ± 0.8	31.3 ± 2.5	25.0 ± 0.2
A23187 (0.5 μM)	42.3 ± 0.1	11.2 ± 1.1	62.4 ± 1.4 *	31.8 ± 1.8

* Lactate dehydrogenase release was 11% or less in each case except as indicated (*) where it was 37%.

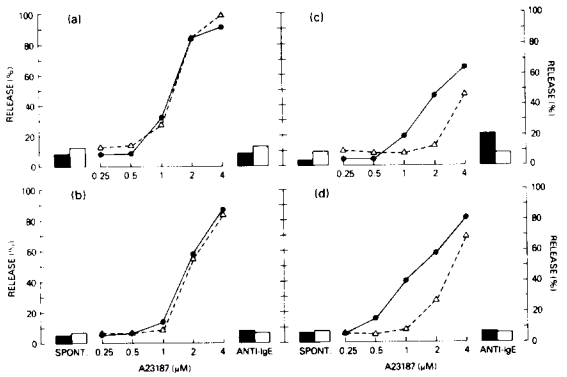

Fig. 3. Response of several variants of the 2H3 subline of rat basophilic leukaemia cells to stimulation with various concentrations of ionophore A23187 or with 2 μg/ml of $F(ab')_2$ fragments of anti-IgE. Release of [^{3}H]serotonin and lactate dehydrogenase are indicated in the same way as in Fig. 1. (a) Variant grown in spinner culture for several months. (b) Cells from the spinner cultures used in (a), 3 days after they had been cultured as monolayers. (c) Cells as in (b), after three once-weekly passages in monolayer culture. (d) Variant which had been routinely passaged in monolayer culture without use of trypsin.

flasks, for several months. When these 'spinner' cells were reintroduced into stationary flasks no appreciable change was observed after 3 days (Fig. 3b). After three once-weekly passages the ability of the cells to respond to anti-IgE was somewhat restored and this was paralleled by a partial recovery of secretion at doses of A23187 which were non-cytotoxic (Fig. 3c).

A second pattern observed with cells unresponsive to stimulation mediated via IgE is illustrated in Fig. 3d. These cells had also been derived from the responsive 2H3 line but had been passaged (in another laboratory) in stationary flasks without use of trypsin (see Materials and Methods). These cells were completely unresponsive to anti-IgE but secreted incorporated serotonin at somewhat high but nevertheless non-cytotoxic doses of ionophore (Fig. 3d). Treatment of these cells with trypsin failed to change their pattern of response.

As a control in each experiment, cells which responded to anti-IgE were examined with the same ionophore preparations. In all cases the separation between the concentrations of ionophore which elicited secretion and those which were cytotoxic was similar to that seen in Fig. 1a. The absolute concentration of A23187 that was cytotoxic to the cells varied somewhat from experiment to experiment. However within an experiment it was virtually identical for all cell lines. This clearly suggested that the cell lines did not differ in sensitivity to the ionophore; merely in their secretory response.

Discussion

The previous failure to elicit non-cytotoxic secretion with the ionophore A23187 from tumour basophils which responded in a Ca^{2+}-dependent manner to triggering via IgE [1] raised the question of whether the tumour cells were utilizing a unique mechanism for secretion. The present results show that reproducible non-cytotoxic secretion can be obtained with ionophores from a subline (2H3) of rat basophilic leukaemia cells which also responds well to triggering via IgE. This response, which was calcium-dependent and occurred at relatively low ionophore concentrations was achieved by increasing the pH of the incubation medium to pH 8. This effect could be due to more efficient but controlled calcium transport at the low ionophore concentrations that were effective at this pH. These findings remove a significant basis for doubt about the similarity of the secretory mechanisms in normal and tumour cells.

High concentrations of ionophores are cytotoxic to most if not all cells; however, tumour basophils appear to be unusually sensitive in this respect. This may be related to the overall energy metabolism of these tumour cells and the way in which they sequester calcium. We previously noted that the tumour cells are remarkably sensitive to antimycin A [2,3]. The latter inhibits IgE-mediated secretion from the tumour cells at doses 100-fold lower than those required to inhibit secretion from normal mast cells [14]. Moreover, at these small doses there is a concomitant increase in spontaneous secretion – a phenomenon not observed with normal mast cells but seen in other Ca^{2+}-dependent secretory phenomena [15]. In the latter instance it has been postulated that the release of Ca^{2+} from mitochondria in metabolically poisoned cells leads to this increase in spontaneous secretion since mitochondrial calcium stores (but not other Ca^{2+} sequestering sites in the cell) are sensitive to inhibitors of oxidative phosphorylation [16,17]. We have suggested that a similar mechanism may pertain in the basophilic leukaemia cells [2,3]

and the finding that removal of extracellular Ca^{2+} had no effect on antimycin A-induced secretion is consistent with this interpretation. Since mitochrondrial calcium accumulation can be driven by exogenous ATP [18] the prevention by glucose of this effect of antimycin A was also to be expected.

A general inhibition of energy-dependent Ca^{2+} sequestering mechanisms in metabolically poisoned cells may also explain why antimycin A potentiates ionophore-induced [^{3}H]serotonin release. In a metabolically active cell these mechanisms are able to cope with a large increase in cytoplasmic Ca^{2+} while in metabolically poisoned cells exposure to A23187 would result in potentially cytotoxic levels of Ca^{2+}. Addition of glucose might support sufficient ATP production to allow sequestering of intracellular Ca^{2+} and thus prevent cytotoxicity, but not enough to permit a level of secretion as great as that induced by ionophore in the absence of antimycin A. The absence of a cytotoxic effect of anti-IgE in metabolically inhibited tumour cells would be expected if anti-IgE induces a relatively small and more physiological calcium influx than A23187. Detailed studies of cellular ATP and Ca^{2+} would be necessary to determine whether this interpretation of our results is correct. Nevertheless, it is clear that with calcium ionophores it is difficult to obtain controlled Ca^{2+} entry and as we have shown (Fig. 1 and Tables I and II) the balance between a physiological response and a cytotoxic effect of A23187-induced Ca^{2+} entry into tumour basophils is a precarious one.

It is interesting to note that the [^{3}H]serotonin release induced by both anti-IgE and A23187 (at both cytotoxic and non-cytotoxic concentrations) shows an absolute dependence on extracellular Ca^{2+} (Table II). This suggests that release of calcium from intracellular stores does not play a role in the secretion induced by these agents.

The effects of changes in culture conditions on the responsiveness of tumour basophils to triggering mediated via IgE or by ionophore (or both), are remarkable. Whether these result from population shifts of cells which differ genetically or simply in their functional status, is unclear. Since the cells used in the present experiments were all derived from a cloned line (2H3) of rat basophilic leukaemia cells and since their responsiveness appeared to show some reversibility, genetic variability seems less likely.

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