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A novel effect of pargyline on cholinergic catecholamine secretion

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Chromaffin cells of the adrenal medulla contain a high concentration of catecholamines, sequestered within secretory granules. Cytosolic catecholamine concentrations are maintained at a very low level by two mechanisms: uptake into the granules via a catecholamine/H^+ carrier; and oxidative deamination by the enzyme monoamine oxidase (MAO; monoamine: oxygen oxidoreductase (deaminating) EC 1.4.3.4), located within the mitochondria. In experiments with isolated cells, the activity of this enzyme released into the medium from damaged cells may confound measurements of secreted catecholamines by converting them to their oxidized metabolites, which may be undetectable. Drugs that inhibit MAO have, therefore, proved useful in preventing metabolism of monoamines such as noradrenaline, the release of which is generally monitored by fluorimetric assay, or by preloading cells with the tritiated form. *N*-Methyl-*N*-2-propynylbenzene methanamine (pargyline), an inhibitor with a degree of selectivity towards MAO-B [1, 2], is a drug that is commonly used to prevent monoamine metabolism in cultured chromaffin cells [3]. Chromaffin cells contain primarily MAO-B [1].

Previous reports have stated that pargyline has no profound effects on synthesis or secretion of catecholamines in cultured pheochromocytoma cells [4] and one would also expect this to extend to primary cultures of chromaffin cells. However, we report here that pargyline does inhibit catecholamine secretion in a dose-dependent manner, and that this may involve non-specific binding of the drug to the acetylcholine receptor.

Materials and methods

Primary culture of bovine adrenal chromaffin cells. Chromaffin cells were isolated from fresh bovine adrenal glands by collagenase digestion, purified on Percoll gradients and cultured according to previously described methods [5]. Cells were transferred from serum-containing medium to a maintenance medium (50% DMEM/50% Ham's F12) after two days and were used for experiments within 4 to 7 days after isolation.

Catecholamine release. Catecholamine release was initiated by replacement of the culture medium with either Locke's buffer or Locke's buffer plus secretagogue [5]. When 50 mM KCl was used to elicit secretion, an equivalent reduction was made in the NaCl content of the buffer; in the case of Ba^{2+}, $CaCl_2$ was replaced by 2 mM $BaCl_2$. After 10 min at room temperature, the supernatant was removed and cells were lysed with Locke's buffer plus 1% Triton X-100. Catecholamine content of both supernatant and cell lysate was determined by the trihydroxyindole method [6]. Results are expressed as the percentage release of total cellular catecholamine content.

Pargyline treatment. Cells were preincubated with pargyline in maintenance medium for 15 min at 37° before culture medium was replaced with Locke's buffer for release measurements. The Locke's buffer contained an equivalent concentration of pargyline, unless otherwise stated.

Measurement of MAO activity. MAO activity was assayed by a modification of the method of Wurtman and Axelrod [7]. Cell lysate was incubated with [^{14}C]tyramine (20 μM) in 0.1 M potassium phosphate buffer, pH 8.0, at 37°. The concentration of endogenous catecholamines in these assays was approximately 20 nM. Oxidation of [^{14}C]tyramine was linear for well over 1 hr under these conditions. The reaction was therefore stopped after 1 hr by addition of an equal volume of 2M HCl. Radioactive aldehyde was extracted into toluene prior to counting in a scintillation counter.

Results and discussion

An inhibitory effect of pargyline was observed when cells preincubated with 0.1 mM pargyline for 15 min were challenged to release catecholamines by exposure to 10 μM nicotine. Over 50% reduction in the percentage of catecholamine released was recorded (Fig. 1).

It was postulated that pargyline might exert its effect via the acetylcholine receptor, either by blocking the receptor itself or by altering its affinity for nicotine by binding to a neighbouring site. Pargyline had no effect on exocytosis stimulated by Ba^{2+} or high K^+, secretagogues which bypass the acetylcholine receptor (Fig. 1), providing evidence for this hypothesis. Furthermore, both the acetylcholine and nicotine-evoked secretion were sensitive to pargyline. 4-Pyridine carboxylic acid 2-(1-methyl-ethyl) hydrazide (iproniazid), a nonspecific MAO inhibitor similar to pargyline, had no effect on receptor-evoked catecholamine secretion (Fig. 1), suggesting that the inhibitory action of pargyline is drug-specific and is independent of its MAO inhibitory action.

The ability of pargyline to inhibit catecholamine secretion in a dose-dependent manner is shown in Fig. 2. Fifty per cent inhibition of nicotine-stimulated catecholamine secretion is achieved with 0.1 mM pargyline, the concentration commonly used with chromaffin cells to give complete inhibition of MAO activity [8]. Although pargyline inhibited both the acetylcholine and nicotinic responses, the latter showed greater sensitivity. The higher dosage of pargyline required to inhibit the acetylcholine response by 50% may be due to the contribution of some muscarinic response in the presence of acetylcholine [9; but see 10, 11].

We investigated the effects of these inhibitors on MAO activity in lysates of whole cells to which trace amounts of [^{14}C]tyramine were added as substrate (Fig. 3). Pargyline is a highly effective inhibitor of chromaffin cell MAO, giving inhibition at concentrations well below the concentration normally used in catecholamine release experiments. Iproniazid, which does not inhibit the secretory response, is a less effective inhibitor by about two orders of magnitude.

Pargyline is known as a suicide inactivator since an irreversible inhibitor of MAO is actually produced by the action of the target enzyme on the drug, which is itself a substrate [12]. In contrast, the ability of pargyline to inhibit catecholamine secretion is completely reversible (Fig. 4), providing further evidence that this effect bears no relation to the MAO inhibitory action of the drug. Full responsiveness of the cells to 10 μM nicotine was restored as soon as pargyline was removed from the medium and no further recovery time was required. The recovery of full secretory activity shows that the integrity of the cells is unaffected by these procedures.

Non-specific effects of pargyline unrelated to its effect on MAO have been reported previously [13]. In the present case, another unpredictable effect of pargyline has been

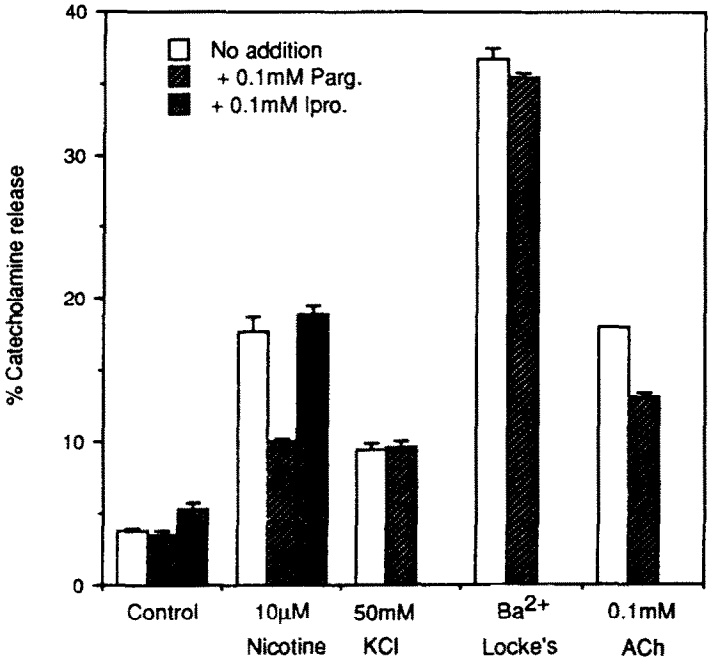

Fig. 1. Effect of MAO inhibitors on exocytosis. The cells were pretreated for 15 min with either 0.1 mM pargyline (Parg.) or 0.1 mM iproniazid (Ipro.) in maintenance medium. The relevant inhibitor was also present in the Locke's buffer. Percentage catecholamine release was measured after 10 min treatment with secretagogue in the presence or absence of the MAO inhibitor. The results shown are means ± SE of triplicate determinations.

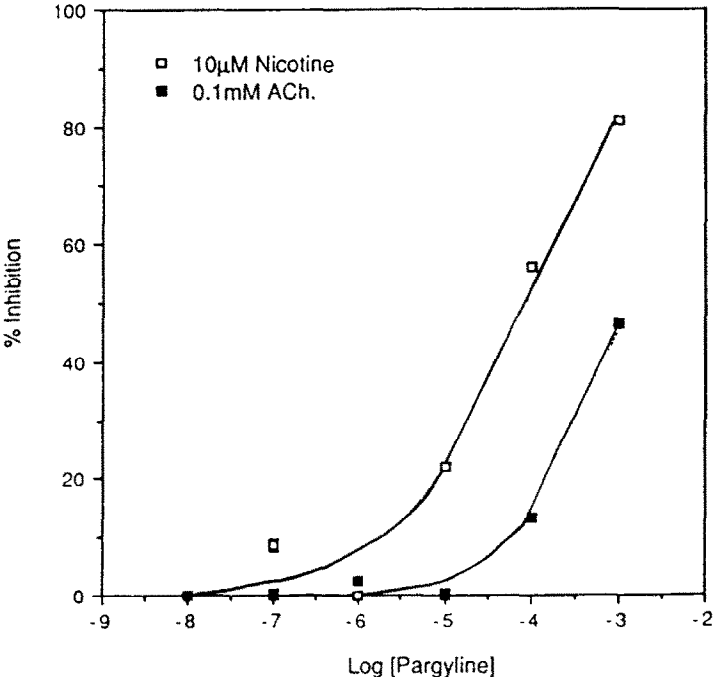

Fig. 2. Inhibition of catecholamine release by various pargyline concentrations. The cells were pretreated for 15 min with a range of pargyline concentrations in maintenance medium. Release was initiated by changing to Locke's buffer or Locke's buffer plus 10 μM nicotine or 0.1 mM acetylcholine (ACh). The results are expressed as percent inhibition, maximum response being 100%, after subtraction of background release. The results shown are means ± SE of triplicate determinations (error bars lie within the symbols used).

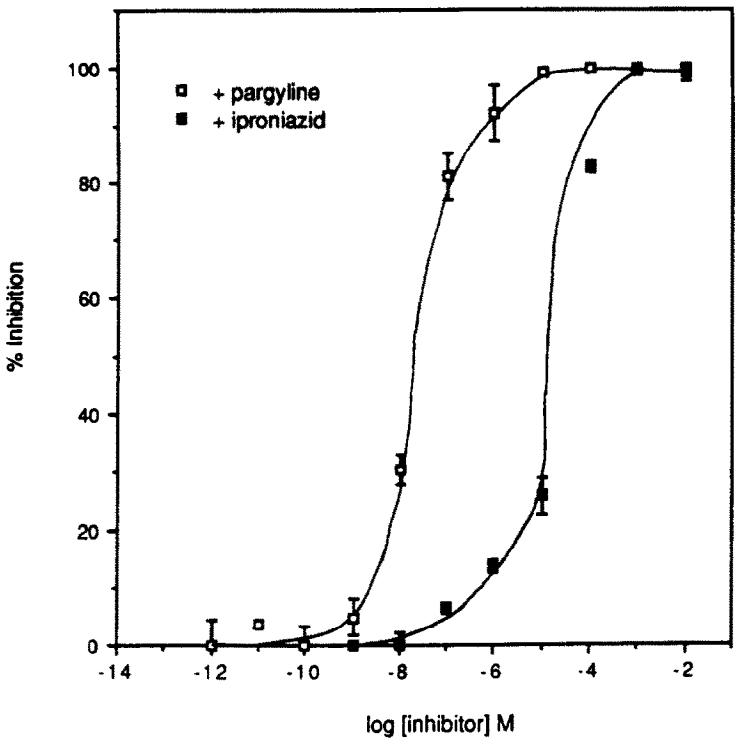

Fig. 3. Inhibition of MAO by pargyline and iproniazid. MAO was assayed as described, in the presence of a range of inhibitor concentrations. Results are expressed as percent inhibition, activity in the absence of inhibitor being 100%. Results shown are means ± SE of triplicate determinations.

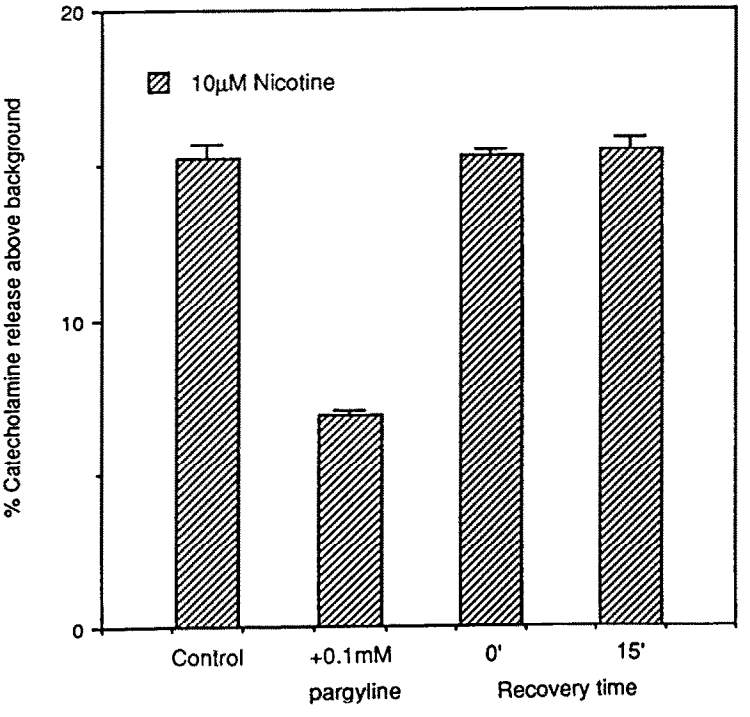

Fig. 4. Recovery of exocytotic response after pargyline treatment. Cells were pretreated with 0.1 mM pargyline for 15 min as described. To measure recovery, cells were pretreated with pargyline for 15 min and then either challenged with 10 μM nicotine in pargyline-free Locke's buffer or allowed to recover in pargyline-free maintenance medium for 15 min at 37° prior to nicotine challenge. Catecholamine release was measured after 10 min treatment with Locke's buffer or Locke's buffer plus secretagogue. The results shown are means ± SE of triplicate determinations after subtraction of background release.

observed, when applied to chromaffin cells, that is distinct from its properties as a MAO inhibitor. Its ability to inhibit catecholamine secretion may be the result of non-specific binding at or near the acetylcholine receptor. This effect may have important implications when using this drug for studying the mechanisms of exocytosis in the chromaffin cell system.

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PAF-Receptors on eosinophils: identification with a novel ligand, [^{3}H]WEB 2086

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The eosinophil has been implicated as a major effector cell in asthma. particularly in causing airway epithelial damage through release of proteins such as the eosinophil cationic protein, major basic protein, and eosinophil peroxidase (EPO) [1]. Platelet activating factor (PAF) has a wide range of biological actions that mimic most of the features of asthma, including bronchoconstriction, increased airway microvascular permeability and protein exudation into the airway lumen [2]. In addition, PAF stimulates eosinophil chemotaxis [3] and release of eosinophil peroxidase [4].

Evidence is accumulating that PAF produces its various effects on target cells through activation of specific membrane receptors. Binding studies with [^{3}H]PAF have, however, often proved difficult as a result of the high level of nonspecific binding and the metabolism and uptake of the radioligand. Therefore, labelled PAF antagonists may represent a more suitable means for probing PAF receptors.

In recent years, several radiolabelled PAF antagonists have been developed. Using [^{3}H]WEB 2086 [5], we have recently been able to identify PAF receptors in intact human platelets [6] and neutrophils [7]. The data reported here describe for the first time the characterization of PAF receptors on eosinophils using [^{3}H]WEB 2086. In addition, we have compared these results with the effect of WEB 2086 on PAF-induced EPO release.

Methods and materials

Eosinophils were obtained by intraperitoneal lavage from polymyxin B-treated guinea pigs [8] and purified using a discontinuous Percoll gradient [9]. The purity of the eosinophils was > 97% with a viability of > 99%. EPO release was measured by a colorimetric assay using 1,2-phenylene-diamine as substrate, as described elsewhere [4]. Binding of [^{3}H]WEB 2086 (specific activity 14 Ci/mmol; Boehringer Ingelheim, F.R.G.) was measured at the indicated concentrations using 1×10^7 eosinophils/ml in duplicates at 25° for 90 min. Nonspecific binding was determined in the presence of either 10 μM WEB 2086 or 1 μM C_{16}-PAF (Bachem, Torrance, U.S.A.) which gave the same results. Bound and free radioligand were separated by rapid filtration and the filters washed twice with 4 ml ice-cold Hepes buffer.

Results

Purified guinea pig eosinophils (2×10^6 cells/sample) were incubated with various concentrations of PAF in the presence and absence of WEB 2086 (200 nM). As illustrated in Fig. 1, PAF induced a concentration-dependent release of EPO from eosinophils with an EC_{50} of 1.2 nM (N = 3). The inactive precursor and metabolite lyso-PAF, however, was ineffective at concentrations up to 10 μM (data not shown). The presence of WEB 2086 caused a parallel rightward shift of the concentration response curve for PAF, indicating competitive antagonism. From this shift an affinity constant (K_B) of 7.3 nM was calculated for the antagonist. In contrast, WEB 2086 did not inhibit enzyme release induced by opsonized zymosan or the calcium ionophore A23187 (Table 1).

Initial attempts with [^{3}H]PAF binding to guinea-pig eosinophils were unsuccessful because of a cellular uptake of the radioligand, giving inconsistent binding and high nonspecific labelling. However, binding of [^{3}H]WEB 2086 to guinea-pig eosinophils was specific, reversible and saturable. A representative saturation isotherm is demonstrated