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SHORT COMMUNICATIONS

Effects of a β-adrenergic blocking agent and isopropylmethoxamine on the release of histamine from guinea pig lung during anaphylaxis *in vitro*

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IT HAS been previously reported that dichloroisoproterenol (DCI), a β-adrenergic blocking agent,[1, 2] attenuates the positive chronotropic and inotropic actions usually observed as a consequence of anaphylaxis *in vitro*[3, 4] in the isolated perfused guinea pig heart. Among the more readily identifiable possible mechanisms of this antianaphylactic action would be an antihistaminic action of DCI or an interference with the binding or release of histamine. The first of these two possibilities has been supported as well as refuted. Mannaioni[5] demonstrated that DCI did not inhibit the effects of exogenous histamine on the perfused guinea pig heart, whereas Subbu *et al.*[6] have shown that naphthylisoproterenol (NI) antagonized the effects of exogenous histamine on the isolated guinea pig auricle.

DCI and some related catecholamines both deplete isolated rat mast cells of histamine[7] and decrease anaphylactic histamine release from perfused sensitized guinea pig hearts.[4] Other investigators have implicated catecholamines in histamine release. Schild[8] has demonstrated that epinephrine blocks antigen-induced histamine release from isolated sensitized guinea pig lung. Later, Koch and Szert[9] found that epinephrine released histamine from perfused rat lung, whereas Mongar and Whelan,[10] employing a number of rat tissues, could not confirm these findings. More recently, Mannaioni *et al.*[4] demonstrated that the presence of norepinephrine in perfused hearts increased antigen-induced histamine release from isolated, perfused, sensitized guinea pig hearts, whereas reserpine pretreatment significantly inhibited. Further, Tidball[11] has shown that epinephrine reverses the glucose-inhibited histamine release from isolated rabbit platelets.

These observations prompted us to investigate the role β-receptor adrenergic blocking agents play in the anaphylactic release of histamine from another organ containing β-adrenergic receptors, i.e. sensitized lung of guinea pig. The compounds studied were NI and isopropylmethoxamine (IMA), an agent[12, 13] which, like NI, prevents the metabolic effects of epinephrine (free fatty acid mobilization and hyperglycemia) while displaying a pharmacological profile which is not parallel to NI.

MATERIAL AND METHODS

Male albino guinea pigs, weighing 250 to 300 g, were obtained from Gordon's Biological Supply Co., Red Bank, N.J., and sensitized by both the i.p. and s.c. administration of 0·5 ml each of a 10% egg albumin (2 × recrystallized, Nutritional Biochemicals Corp.) suspension. Four to six weeks later, the guinea pigs were killed by a light blow over the head.

The lungs were removed, washed, and sliced with a Stadie-Riggs tissue slicer. The slices were further washed four times with oxygenated, iced Tyrode's solution (pH 7·4) containing glucose, and the slices weighed. The weighed lung slices (~ 100 mg) were placed in a beaker containing 6 ml of Tyrode's solution, or the solution containing drug, and incubated at 37° for 15 min. At this time the challenge (2 ml of a 12 mg egg albumin suspension/ml) was added and incubation continued for a further 15-min period. The tissue histamine was then extracted by boiling the minced slices in 5 ml of 1 N HCl and the histamine determined in an aliquot of the neutralized acid by the method of Shore *et al.*[14] with a Farrand spectrofluorometer with an excitation wavelength of 360 mμ and fluorescence wavelength of 460 mμ (uncorrected). Histamine content of different slices from the same lung did not vary more than five % as determined by this method. The naphthylisoproterenol (NI) variously termed Nethalide or alderlin was kindly supplied by Dr. J. Black; ICI and the isopropylmethoxamine or B.W. 61-43 (IMA) was a gift from Dr. J. J. Burns (Burroughs-Wellcome). Neither NI nor IMA, at the concentrations employed in these studies, interfered with the fluorometric determination of histamine.

RESULTS

At final concentrations of 3 and 1 mM, IMA itself did not provoke significant histamine release (compare the histamine levels of unchallenged control and unchallenged IMA, line 1 vs. lines 3 and 5, Table 1.) These results, as well as the subsequent findings reported below, are calculated on the

TABLE 1. THE EFFECT OF ISOPROPYLMETHOXAMINE (IMA) AND NAPTHYLISOPROTERENOL (NI) ON ANAPHYLACTIC RELEASE OF HISTAMINE FROM GUINEA PIG LUNG SLICES

	Drug	Concentration (mM)	R_x	Histamine μg/g wet wt.; mean ± S.E.)	Tests of significance
1.			Control	44·3 ± 6·2 (6)†	1 vs. 3, 1 vs. 5 NSD‡
2.			Challenge*	30·0 ± 2·1 (6)	
			Difference (Δ)	14·3 ± 1·2	Δ 1 > 2, P 0·01
3.	IMA	3	Control	38·1 ± 7·0 (6)	
4.	IMA	3	Challenge	37·1 ± 7·2 (6)	
			Δ	1·0 ± 0·2	Δ 3 vs. 4, NSD
5.	IMA	1	Control	38·2 ± 6·4 (6)	
6.	IMA	1	Challenge	35·3 ± 6·6 (6)	
			Δ	2·9 ± 0·6	Δ 5 vs. 6, NSD
7.			Control	28·9 ± 5·1 (8)	7 > 9, P 0·01 7 > 11, P 0·05
8.			Challenge	20·7 ± 4·0 (8)	
			Δ	8·2 ± 1·6	Δ 7 > 8, P 0·01
9.	NI	3	Control	7·5 ± 0·5 (3)	
10.	NI	3	Challenge	8·2 ± 1·8 (3)	
			Δ	−0·7 ± 0·1	Δ 9 vs. 10, NSD
11.	NI	1	Control	21·8 ± 3·2 (6)	
12.	NI	1	Challenge	24·0 ± 3·8 (6)	
			Δ	−2·2 ± 0·4	Δ 11 vs. 12, NSD
13.	NI	0·1	Control	30·8 ± 5·1 (5)	
14.	NI	0·1	Challenge	26·0 ± 5·7 (5)	
			Δ	4·8 ± 0·6	Δ 13 > 14, P 0·01

* See Methods.

† Number of experiments per mean. Each experiment consisted of two separate determinations.

‡ No significant difference.

basis of the wet weight of the lung slice at the start of the incubation period. Consequently, any increase in water content of the slice during incubation would not influence the calculations. In contrast, it was clearly demonstrated that at a final concentration of 3 mM, NI provokes histamine release (Table 1, line 7 vs. line 9). At one third this concentration, the histamine-releasing properties of NI are less apparent but nevertheless significant (line 7 vs. 11) whereas at 0·1 mM, there is no histamine release due to NI (line 7 vs. 13).

The antigen challenge of the control Tyrode slices resulted in a mean histamine release of 32% (line 1 vs. 2, Table 1) and 28% (line 7 vs. 8). In the presence of 1 and 3 mM NI, this antigen-induced histamine release was prevented. The apparent NI blockade of antigen-induced histamine release at final concentrations of 3 mM and 1 mM (lines 9 vs. 10 and 11 vs. 12) may reflect the intrinsic release of histamine as a consequence of NI itself, which rendered the tissue incapable of responding to antigen challenge. This is unlikely since the histamine levels of the lung, after exposure to 1 mM, were severalfold greater than the levels observed with 3 mM, but neither responded to antigen challenge. Antigen-induced histamine release, however, was diminished by NI at a concentration of NI that did not cause intrinisc histamine release (line 13 vs. 14). In parallel experiments it was demonstrated that at concentrations of 3 mM and 1 mM, IMA significantly inhibited antigen-induced histamine release in the absence of a significant intrinsic action of IMA (lines 3 vs. 4 and 5 vsl 6).

DISCUSSION

The histamine-releasing capacity of the β-receptor antagonist DCI has been demonstrated[7] in cells of rat peritoneal fluid. The present investigations have demonstrated that another β-receptor antagonist, NI, elicits nonantigenic histamine release from guinea pig lung slices incubated in a physiological medium. In contrast, IMA, a drug that antagonized the metabolic effects of catecholamines, but which is pharmacologically unlike β-receptor antagonists, does not itself induce significant release. However, dichloroepinephrine and dichloroarterenol[7] were as active as DCI in releasing histamine but were less active as β-receptor antagonists.[15] Consequently, it would appear unlikely that the histamine-releasing properties of these agents are related to their ability to inhibit the β-pharmacological actions of epinephrine.

The histamine release that results from antigen challenge of sensitized, perfused guinea pig hearts and its blockade by DCI and NI, first reported by Mannaioni *et al.*[4] has been extended in the present studies with NI and IMA in lung slices obtained from sensitized guinea pigs. Of interest is the relatively potent blocking action of IMA, since IMA is a fairly specific antagonist of epinephrine's metabolic effects.[12, 13] Thus, IMA resembles NI in blocking histamine release and the metabolic effects of catecholamines, but it does not display a cardiovascular profile similar to NI. The effectiveness of reserpine and β-adrenergic blockers in attenuating histamine release from the perfused guinea pig heart[4] and the restoration of histamine release by norepinephrine infusion tend to support the conclusion of Mannaioni *et al.*[4] that catecholamines modulate histamine release during anaphylaxis. It is suggested that a similar adrenergic-dependent mechanism modulates the ability of NI and IMA to inhibit antigen-induced histamine release from guinea pig lung slices *in vitro*. Perhaps the unifying concept to explain the various observations discussed above is that sufficient metabolic activity is necessary to maintain the energy levels required for histamine release in the face of an appropriate challenge. Diamont and Ůvnas[16] presented evidence for an obligatory energy for histamine release, These workers showed that this requirement could be fulfilled even in anoxic situations *in vitro* if sufficient glucose were present to maintain energy production through anaerobic glycolysis. Since the catecholamines[17] facilitate eventual conversion of glucose to lactic acid, which yields concomitant amounts of energy, drugs that inhibit or shift this metabolic pattern (NI, DCI, or IMA) by antagonizing catecholamines should prevent adequate energy production and the release of histamine. The potent histamine-releasing action of NI *in vitro* may be related to its catecholamine-like intrinsic action.[18] Further, until the concentrations employed in these studies *in vitro* are achieved *in vivo*, it will not be definitely established whether NI will elicit an anaphylactoid response in the animal.

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