acid hydrolysis of the inhibited enzyme and conversion of the constitutent amino-acids to their ethyl esters. Mass spectrometry of the esterified mixture at 70 eV gave a scan which showed ions corresponding to all mass numbers up to mass 300, an indication that low molecular weight peptides were present in the mixture. Precise mass measurements of the ion at mass 187 gave a mass of 187·1444 ± 0·0009 for this ion which corresponded to the expected fragment ion (Biemann, Seibl & Gapp, 1961) $\overset{+}{H_2N} = CH\cdot(CH_2)_4\cdot NH\cdot CH_2\cdot CO_2Et$, (187·1446) derived from a ϵ-carboxy-methyl lysine residue. The product from the reaction conducted at pH 5·6 when treated in a similar manner showed an ion at mass 196 which at high resolution was resolved into two ions, mass 196·0974 ± 0·0010 ($C_{10}H_{14}NO_3$) and 196·1082 ± 0·0010 ($C_9H_{14}N_3O_2$, 196·1086). The latter ion corresponded to the expected ion, $EtO_2C\cdot CH_2C_3H_2N_2-CH_2\cdot CH=\overset{+}{N}H_2$, derived from N-carboxymethylhistidine.

A control sample of ribonuclease, which had not been reacted with iodoacetate, when treated in a similar manner showed the presence of only traces of the relevant ions.

Welsh School of Pharmacy,
University of Wales Institute of Science and Technology,
Cathays Park,
Cardiff, U.K.

S. I. AHMAD
H. J. SMITH

July 23, 1973

REFERENCES

AL SHABIBI, H. (1972). Ph.D. thesis, University of Wales.
BIEMANN, K., SEIBL, J. & GAPP, F. (1961). *J. Am. chem. Soc.*, **83**, 3795–3804.
GUNDLACH, H. G., STEIN, H. & MOORE, S. (1959). *J. biol. Chem.*, **234**, 1754–1760.
LAWSON, W. B. & SCHRAMM, H. J. (1962). *J. Am. chem. Soc.*, **84**, 2017–2018.
SCHOELLMANN, G. & SHAW, E. (1963). *Biochemistry*, **2**, 252–255.

Chemical antagonism by iodine of the pharmacological activity of some sympathomimetic amines

In a series of experiments concerning the dependence of β-adrenoceptor-mediated effects on cellular metabolism, it was found that inhibition of pendular movements of the rabbit isolated intestine, produced by isoprenaline, was prevented by iodoacetate (Hall, 1971). When pyruvate was added to the organ bath in the continued presence of iodoacetate, the inhibitory response to isoprenaline was restored. These experiments were interpreted in support of the concept that β-adrenoceptor-mediated effects are dependent on the integrity of cellular glycolytic pathways. In a more recent series of experiments attempts were made to repeat the observation using a sample of iodoacetate from a different commercial source. The new sample was without effect on isoprenaline responses. Fresh samples of iodoacetate from both suppliers were then compared. Those from the one supplier always blocked responses to isoprenaline, while those from the other did not. Chemical analysis showed that the active samples of iodoacetate contained about 0·6% free iodine. The activity of the active iodoacetate solutions now appears to have been due to their content of free iodine.

Experiments were made on segments of rabbit small intestine and on the rat portal vein. All tissues were suspended in Krebs solution at 37°, and gassed with 5% CO_2

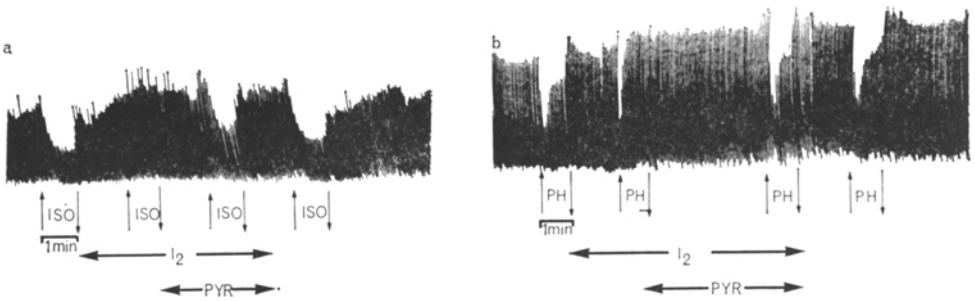

FIG. 1(a). The inhibitory effect of iodine (30 μM at I_2) on responses of the rabbit isolated small intestine to isoprenaline (0·047 μM; 10 ng ml^{-1} at ISO). Pyruvate (10 mM at PYR) prevented this action of iodine.

(b). Iodine (30 μM at I_2) did not abolish the inhibition of spontaneous activity produced by phenylephrine (0·598 μM; 0·1 μg ml^{-1} at PH) but the duration of the response was curtailed. The response to phenylephrine was restored to normal by the presence of pyruvate (10 mM at PYR).

in O_2. Recordings were made on a kymograph with either an isotonic frontal writing lever or with an isometric dynamometer (Ugo Basile Instruments).

Fig. 1 illustrates the effects of iodine (30 μM; 4 μg ml^{-1}) in alcoholic solution and pyruvate (10 mM; 0·88 mg ml^{-1}) on the inhibitory responses to isoprenaline (10 ng ml^{-1}; 0·047 μM) and phenylephrine (0·1 μg ml^{-1}; 0·598 μM) in the rabbit intestine. The inhibitory response to isoprenaline was abolished in the presence of iodine, but was restored (without removing the iodine) when pyruvate was added to the organ bath. Similar results were obtained with iodine (30 μM) in 10% potassium iodide solution.

The inhibitory response to α-adrenoceptor agonists, such as phenylephrine, has two components (Fig. 1b)—an initial inhibition of rapid onset, succeeded by a slow recovery towards control despite the continued presence of the drug, and there is often an overshoot on washout (Bowman & Hall, 1970). In the presence of iodine (30 μM), the initial rapid inhibition produced by phenylephrine was still present. However, the secondary phase of recovery was enhanced to the extent that complete return to the control amplitude of contractions occurred during the continued presence of phenylephrine, so that the overall duration, but not the extent, of the inhibitory response was curtailed. Pyruvate restored the response to phenylephrine to the form of the control response. Very high concentrations of iodine (150–230 μM) did slightly reduce the peak inhibitory response to phenylephrine but it was never abolished. The diluents for iodine (20% alcoholic saline or 10% KI) were without effect on responses to sympathomimetic amines. The effects of iodine (30 μM) were identical with those produced by the samples of iodoacetate found to contain about the same concentration of free iodine (Hall, 1971), and the effects of these iodoacetate solutions must therefore be attributed to this contaminant.

β-Adrenoceptor mediated inhibitory responses to orciprenaline (1–5 μg ml^{-1}; 4·7–23·5 μM), salbutamol (1–5 μg ml^{-1}; 4·2–21 μM) and AQL 208* (10–20 ng ml^{-1}; 0·029–0·058 μM) were blocked by iodine (30–75 μM) and restored by pyruvate (10mM) in the same way as were responses to isoprenaline. Another keto-acid, acetoacetate (10 mM) behaved like pyruvate in its ability to restore responses that had been abolished by iodine but the two hydroxy acids, lactate (10–40 mM) and β-hydroxybutyrate (10–40 mM) were ineffective in this respect. α-Adrenoceptor-mediated inhibitory responses to metaraminol (2 μg ml^{-1}; 12 μM) were modified by iodine and pyruvate in the same way as were responses to phenylephrine. However, α-adrenoceptor-mediated responses to methoxamine (0·15–0·3 μg ml^{-1}; 0·7–1·4 μM) were completely

* L-1-(3,4,5-trimethoxybenzyl)-6,7-dehydroxy-1,2,3,4-tetrahydroquinoline HCl.

unaffected by iodine even in concentrations up to 312 μM, the highest concentration tested. Both the α- and β-adrenoceptor effects of adrenaline and noradrenaline in the rabbit small intestine (Bowman & Hall, 1970) were abolished by iodine and restored by pyruvate or acetoacetate but not by lactate nor β-hydroxybutyrate. To test whether there was a chemical interaction between iodine and the sympathomimetic amines, they were mixed before addition to the bath and excess iodine removed by titration with sodium thiosulphate. No response of the tissue was obtained with the reaction mixtures except with methoxamine where the reaction mixture produced a response identical to that of the control concentration of methoxamine.

As in the rabbit intestine, the β-adrenoceptor-mediated inhibitory responses of the rat portal vein to isoprenaline (0·47 μM), salbutamol (1·67 μM), orciprenaline (0·94 μM) and AQL 208 (0·87 μM) were abolished by iodine (30 μM) and restored by pyruvate (10 mM) or acetoacetate (10 mM) but not by lactate (10–40 mM) nor by β-hydroxybutyrate (10–40 mM). α-Adrenoceptor-mediated excitatory effects on the rat portal vein produced by adrenaline, noradrenaline, and, surprisingly, by phenylephrine and metaraminol were also abolished by iodine (30–75 μM) and restored by pyruvate (10 mM). The α-adrenoceptor-mediated response to methoxamine was unaffected by iodine in concentrations up to 312 μM.

The ability of iodine to abolish the response of a sympathomimetic amine, and of pyruvate to restore it, did not, as was initially supposed, depend upon interaction with a particular receptor type (i.e. α- or β-adrenoceptors), because responses of the same tissue mediated by different types of receptor could be abolished. Nor did it depend upon the nature of the response (excitatory or inhibitory) because both types of response, whether in the same tissue (rat portal vein) or in different tissues, could be abolished. The lack of activity of amine-iodine mixtures appears to be the result of a chemical interaction; the iodide ion, as in control potassium iodide solutions, did not share this ability to interact with the amines.

α-Adrenoceptor-mediated *excitatory* effects in the rat portal vein produced by phenylephrine and metaraminol were totally abolished by iodine, whereas their α-adrenoceptor-mediated *inhibitory* effects in the rabbit intestine were reduced only in duration, findings which are difficult to interpret.

These experiments stress the care necessary when using commercially available samples of iodoacetate, and offer an interesting example of chemical antagonism of pharmacological activity.

The metaraminol (Aramine) was kindly supplied by Dr. F. G. Clayton of Merck, Sharp & Dohme.

Department of Pharmacology MOIRA T. HALL
University of Strathclyde,
George Street,
Glasgow, G1 1XW, U.K.

May 16, 1973

REFERENCES

BOWMAN, W. C. & HALL, M. T. (1970). *Br. J. Pharmac.*, **38**, 399–415.
HALL, M. (1971). *J. Pharm. Pharmac.*, **23**, *Suppl*, 229S.