Table 2. IAA concentration in plasma and erythrocytes after i.p. injection of L-Try (0.3 mmoles/kg)

Concentration of IAA (nmoles/ml)	Time (h) after injection of L-Try 0	0.5	1	3	6
Plasma	0.06	1.20±0.40	1.08±0.17	0.80±0.23	0.51±0.11
Erythrocytes	0.06	0.91±0.34	0.74±0.11	0.46±0.06	0.63±0.17

* ±SE of 5 determinations.

vent mixtures; n-BuOH:CH_3COOH:H_2O (40:10:15, v/v) and methylacetate:propyl alcohol:25% NH_4OH (45:35:20, v/v). The R_f values of authentic IAA and IAAsp were 0.76 and 0.50 with the former solvent, and 0.36 and 0.05 with the latter, respectively. The regions equivalent to the R_f values of IAA and IAAsp of the chromatogram were extracted with 10 ml of 80% ethanol aqueous solution 3 times and the extracted solution was evaporated to dryness at 40 °C. The residue was dried well under a stream of N_2 and 50 µl of trifluoroacetic anhydride (TFAA) was added to the residue and allowed to react for 60 min at 48 °C. Excessive TFAA was removed with a stream of N_2 and the trifluoroacetylated sample was dissolved in 1 ml of methanol containing 10 ng of α-benzene hexachloride (α-BHC). 1 µl of the solution was used for gas chromatographic analysis with a Shimazu Model GC-4BPF gas chromatograph equipped with an electron capture detecter and fitted with a 200 cm glass column, 0.3 cm in diameter. The column packing consisted of 1.5% silicon OV-17 on Gas-Chromosolve W. The column temperature was 200 °C, and the determination temperature was 250 °C. N_2 (40 ml/min) was used as carrier gas.

Results and discussion. When 500 mg (2.85 mmoles) of IAA per kg animal was injected i.p., 78.20±12.59 µmoles of IAA and 0.009±0.002 µmoles of IAAsp were detected in urine samples within 24 h, although none of them was found in the urine of the control animals treated with no IAA. Furthermore, when IAA and aspartic acid (Asp) were simultaneously given, i.p. in the former and s.c. in the latter, IAA and IAAsp were detected more, but when Asp was exclusively applied, they were not detected, as shown in table 1. Weissbach et al.[5] reported that IAA was formed from L-tryptophan enzymatically by guinea-pig kidney and liver extracts, and also by intestinal bacteria. Urinary excretion of IAA was observed by Gordon et al.[7] in germ-free mice to which L-tryptophan was given i.p. However, the formation of IAAsp has never been observed in IAA- or tryptophan-treated animals. In the present experiment, 0.3 mmoles (61.2 mg)/kg of L-tryptophan was injected i.p., IAA was found in both plasma and erythrocytes of the treated rats 30 min after application, but IAAsp was not detected as shown in table 2. In 24-h urine samples, after the application of tryptophan, IAA was detected in quantities as much as 0.088±0.009 µmoles/animal, and IAAsp was not found by the method used, although very small amounts of IAAsp might be present. Further studies on the significance of IAAsp formation are now in progress.

1 Present address: Research Laboratories, Tanabe Pharmaceutical Co. Ltd, No. 16-89, Kashima 3-chome, Osaka, 532 (Japan).
2 W.A. Andreae and N.E. Good, Pl. Physiol. *30*, 380 (1955).
3 E. Tillberg, Physiol. Pl. *31*, 271 (1974).
4 E. Epstein and S. Lavee, J. exp. Bot. *28*, 629 (1977).
5 H. Weissbach, W. King, A. Sjoerdsma and S. Udenfriend, J. biol. Chem. *234*, 81 (1959).
6 S.D. Seely and L.E. Powell, Analyt. Biochem. *58*, 39 (1974).
7 S.A. Gordon, R.J.M. Fry and S. Barr, Am. J. Physiol. *222*, 399 (1972).

Studies on the role of the mast cell in local calcergy

J. McClure

Department of Pathology, The Queen's University of Belfast, Belfast (N. Ireland), 9 May 1979

Summary. The treatment of animals with disodium chromoglycate and/or cytochalasins which inhibit mast cell degranulation has no influence on the development of local calcergy induced in the mouse by the s.c. injection of lead acetate.

In a study of the pathogenesis of local calcergy it has been reported by Bridges and McClure[1] that 5 h after the injection of the lead acetate the dermal mast cells have degranulated and the adjacent capillaries are dilated and surrounded by deposits of calcium and phosphate ions. In order to explore the role of the mast cell in this reaction, experiments were performed in which animals were given s.c. injections of lead acetate and also treated with substances known to stabilize mast cells and inhibit their degranulation.

A total of 201 male adult white mice was used (mean weight 30±2.1 g) and 4 experimental procedures were performed. In the 1st experiment 15 animals were used in 3 groups of 5. Each of the 1st group was given a single s.c. injection of lead acetate (PbAc) in 0.1 ml of solution into the lumbar region. The 2nd group was given similar injections and in addition, each animal was given an i.p. injection of 0.3 mg of DSCG (disodium chromoglycate, 'Intal', Fisons Ltd, Leics., England) in 0.1 ml of solution (10 mg/kg b. wt) immediately after the s.c. injection. The 3rd group was similarly treated except that the i.p. dose of DSCG was 3 mg/0.1 ml (100 mg/kg b. wt).

In the 2nd experiment 42 mice were used in 14 groups of 3. Each animal was given an s.c. injection of lead acetate and groups of animals were given either 0.3 mg DSCG/0.1 ml or 3 mg DSCG/0.1 ml by i.p. injection. These latter injections were given 1 h before and 1, 2, 3, 4, 5, 6, and 8 h after the injection of lead acetate.

In the 3rd experiment 72 mice were used in 18 groups of 4. Each animal was injected with PbAc 100 µg/0.1 ml into the lumbar region. Groups of animals were injected (into the same site as PbAc) with cytochalasin A (extract of *Helminthosporium dermatoideum*, Sigma Chemical Co., St. Louis, Mo.). This was made up in twice deionized water in 2

concentrations (1 µg/0.1 ml and 10 µg/0.1 ml). Groups of animals were given 1 of these doses into the same injection site as PbAc 1 h before, at the same time as, 1, 2, 3, 4, 5, 6, and 8 h after the PbAc.

In the 4th experiment the effect of the combined administration of DSCG and cytochalasin A was tested in 72 mice divided into 18 groups of 4. Each animal was injected with PbAc 100 µg/0.1 ml into the lumbar region. Groups of animals were injected with both cytochalasin A (s.c. into the same site as PbAc) and DSCG (by the i.p. route) in the dose combinations 1 µg/0.1 ml s.c. and 0.3 mg/0.1 i.p. or 10 µg/0.1 ml s.c. and 3 mg/0.1 ml i.p. These combinations were injected 1 h before, at the same time as, 1, 2, 3, 4, 5, 6, and 8 h after the injection of PbAc.

In each experiment the s.c. injection sites were recovered after 7 days. Each site was X-rayed with a Faxitron cabinet X-ray machine and studied histologically and histochemically. The extent of the calcific reaction was determined by the chloranilic acid staining reaction for calcium (Eisenstein et al.[2]) and the von Kossa technique for phosphate.

Results. The injection of PbAc 100 µg/0.1 ml resulted in calcification in the control animals. This was macroscopically visible after 7 days and confirmed radiologically and histochemically. Animals which had been injected at the same time with DSCG (in either dose) showed identical areas of calcification. None of the drug treatments had any effect on development of local calcergy. Calcification occurred which was identical in extent and distribution to that found in the control animals.

Discussion. It has been shown that substances are released from rodent mast cells by 2 mechanisms one being a nonselective release of amine as a result of lysis of the cell membrane which is blocked by DSCG (Orr and Cox[3], Orr et al.[4], Orr et al.[5]) and the other a selective release of granules through a process of exocytosis (Ellis et al.[6]) which is mediated by microfilaments and blocked by cytochalasins (Orr et al.[7]). These separate mechanisms were blocked separately and in combination by the drug treatments used in the present experiments and none of these influenced the calcification reaction induced by PbAc and the reactions were indistinguishable from those in animals which had been injected with PbAc only.

In their study of local calcergy in the rat, Selye et al.[8] had suggested that discharged mast cell granules attracted lead, calcium and phosphate and that the resulting complex acted as a nidus which initiated a calcification reaction. Bridges and McClure[1] were unable to find any evidence in their study of local calcergy in the mouse to support this theory and instead suggested that mast cell degranulation played a nonessential role in the reaction being only associated with vascular dilatation and increased permeability.

In the present experiments the prevention of mast cell degranulation did not affect the calcergic reaction and therefore these results support the suggestion by Bridges and McClure[1] that the mast cell does not play an essential role in the pathogenesis of local calcergy in the mouse.

1 J.B. Bridges and J. McClure, Calcif. Tissue Res. *10*, 136 (1972).
2 R. Eisenstein, M. Werner, S. Papajiannis, W. Konetzki and I. Laing, J. Histochem. Cytochem. *9*, 154 (1961).
3 T.S.C. Orr and J.S.G. Cox, Nature *223*, 197 (1969).
4 T.S.C, Orr, J.M. Gwilliam and J.S.G. Cox, Immunology *19*, 469 (1970).
5 T.S.C. Orr, J.M. Gwilliam and J.S.G. Cox, Immunology *21*, 405 (1971).
6 H.V. Ellis, A.R. Johnson and N.C. Moran, J. Pharmac. exp. Ther. *175*, 627 (1971).
7 T.S.C. Orr, D.E. Hall and A.C. Allison, Nature *236*, 350 (1972).
8 H. Selye, G. Gabbiani and N. Serafimov, J. Histochem. Cytochem. *12*, 563 (1964).

Influence of clonidine on experimental hypertension induced by cholinergic stimulation

J.J. Buccafusco and S. Spector

Roche Institute of Molecular Biology, Nutley (New Jersey 07110, USA), 20 August 1979

Summary. Hypertension may be induced by pharmacologic activation of central cholinergic receptors either indirectly, through the injection i.v. of physostigmine, or directly, through the injection i.v. of arecholine in anesthetized rats. Activation of peripheral preganglionic cholinergic receptors with dimethylphenylpiperazinium iodide (DMPP) also produced a hypertensive response. Pretreatment with various doses of clonidine caused inhibition of the pressor response to central cholinergic stimulation but was without effect on the response to ganglionic cholinergic stimulation.

Peripheral[1] or central[2] injection of physostigmine elicits a rise in arterial blood pressure (BP), and this response is mediated through the release of endogenous brain acetylcholine (ACh) acting on muscarinic receptors. Centrally acting muscarinic receptor agonists, such as oxotremorine or arecholine, can activate this central cholinergic pathway by directly stimulating muscarinic receptors[3,4]. This elevation in BP is due to an increase in sympathetic nerve activity following central cholinergic stimulation[2,5]. We have recently reported[6] that administration i.v. of 100 µg of clonidine blocks the release of ACh from certain brain regions and as a result produces marked inhibition of the pressor response produced by injection i.v. of several doses of physostigmine. The present study was undertaken to elucidate the nature of the interaction between clonidine and the function of central and peripheral cholinergic neurons in the regulation of BP, and to determine whether this model of central cholinergic hypertension could be used to evaluate new antihypertensive drugs.

Male, Wistar rats weighing 250–400 g were anesthetized with urethane (1.3 g/kg, i.p.). Heparinized saline-filled polyethylene catheters were inserted into the left common carotid artery and left jugular vein for the direct recording of BP and injection i.v. of drugs, respectively. Drugs were dissolved in saline and injected in a volume of 0.1 ml/100 g b.wt. All drug weights refer to the salt. The basal level of mean arterial pressure (MAP) in 60 anesthetized rats averaged 74±2 mm Hg (mean ±SEM). Rats were initially injected with a control dose of cholinergic agonist, and after return of BP to preinjection levels, animals received clonidine. 30 min later a 2nd dose of agonist was administered. Preliminary experiments showed that all responses to cholinergic agonists were reproducible after consecutive injections spaced 30 min apart. When arecholine was used,