radioactive peaks. The fastest-moving component had an apparent molecular weight of about 23,000 while a larger, less mobile peak had a M.W. in the region of 50,000 and could be a dimer of the smaller component. The third peak was found at the origin and varied considerably in size. It seems likely that this peak was due to aggregated material since it could be reduced considerably by prior treatment of the sample with the reducing agent dithiothreitol.

The results suggest that BCM is selectively bound to one or more protein components of smooth muscle cell membranes. The inhibition of this binding by low concentrations of atropine indicates that it may be related to muscarinic receptors.

This work was supported by a grant from the Medical Research Council. C.M.S.F. is an M.R.C. Scholar.

REFERENCES

ELLMAN, G. L., COURTNEY, K. D., ANDRES, V. & FEATHERSTONE, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.*, **7**, 88–95.

GILL, E. W. & RANG, H. P. (1966). An alkylating derivative of benzilylcholine with specific and long-lasting parasympatholytic activity. *Mol. Pharmac.*, **2**, 284–297.

IPATA, P. L. (1967). A coupled optical enzyme assay for 5′-nucleotidase. *Analyt. Biochem.*, **20**, 30–36.

MAIZEL, J. V. (1969). Acrylamide gel electrophoresis of proteins and nucleic acids. In: *Fundamental Techniques in Virology*, ed. Habel, K. & Salzman, N. P. pp. 334–362. New York: Academic Press.

RANG, H. P. (1967). The uptake of atropine and related compounds by smooth muscle. *Ann. N.Y. Acad. Sci.*, **144**, 756–767.

SONG, C. S. & BODANSKY, O. (1967). Subcellular localization and properties of 5′-nucleotidase in the rat liver. *J. biol. Chem.*, **242**, 694–699.

Specific inhibitors for angiotensin II and angiotensin I

W. K. PARK and D. REGOLI*

Department of Pharmacology, University of Sherbrooke, Canada

Substitution of an unnatural amino-acid (Acpc: 1-Aminocyclo-pentanecarboxylic acid) for each of the eight amino-acids of the angiotensin molecule shows that 6-His and 8-Phe are essential for the activities of angiotensin II (AII) and angiotensin I (AI) on the arterial pressure (nephrectomized rats anaesthetized with urethane 1·4 g/kg s.c.) and on the rat isolated colon (Regoli & Vane, 1964).

Moreover, 8-Acpc AII, but not 6-Acpc AII antagonized the activities of AII and of AI on the two preparations.

Various analogues of angiotensin II substituted in position 8 have been tested for their inhibitory activities against AII and AI *in vivo* (rat blood pressure) and *in vitro* (rat isolated colon) (Table I).

TABLE 1. *Effect of 8 substituted angiotensin analogues on the pressor and myotropic (rat colon) action of* AII *and* AI

		Rat arterial pressure		Rat isolated colon	
Antagonist (Ant.)	Agonist (A)	Ratio Ant./A†	Inhibition %	Ratio Ant./A	Inhibition %
8 Acpc AII	AII	50	65±7	250	67±8
	AI	25	70±10	10	71±12
8 Ala AII	AII	50	60±5	125	82±7
(Park *et al.*, 1967)	AI	25	68±7	10	75±5
8 D-Phe AII	AII	100	52±8	500	50±11
	AI	50	50±11	20	48±9
8 Achc AII‡	AII	100	50±6	500–1000	70±13
	AI	50	53±5	20–40	65±11
4 Phe-8 Tyr AII	AII	50	58±10	5000	64±14
(Marshall *et al.*, 1970)	AI	25	40±7	100	20±7

Means of six experiments †Ratio for *in vivo* experiment has been calculated by dividing the dose of antagonist given by infusion ((μg/kg)min) by the dose of agonist given by injection (ng/kg). ‡Achc: A-Aminocyclohexanecarboxylic acid.

The specificity of the antagonism for angiotensin has been assessed by measuring the effect of the 8 substituted angiotensins against AII, AI, and noradrenaline (NA) on the rat blood pressure, AII, AI, acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) on the rat isolated colon. The 8 substituted analogues of AII antagonize the pressor effects of AII and AI and slightly that of NA, but do not influence the myotropic effect of ACh and 5-HT on the rat isolated colon.

It is proposed that: (a) 6-His is essential for binding angiotensin to the receptor and an aromatic ring in position 8 is necessary for pressor and myotropic activities, (b) AI acts on the same receptor as AII, (c) 8 substituted analogues of AII may fulfil the criteria for competitive inhibitors, because inhibition is reversible and the extent of the inhibition depends on the relative concentration of agonist and antagonist.

REFERENCES

MARSHALL, G. R., VINE, W. & NEEDLEMAN, P. (1970). A specific competitive inhibitor of angiotensin II. *Proc. Nat. Acad. Sci. U.S.A.*, **67**, 1624–1630.

PARK, W. K., SMEBY, R. R. & BUMPUS, F. M. (1967). Synthesis of [5-isoleucine, 8-alanine]-angiotensin II by the solution method synthesis and the solid-phase method synthesis. *Biochemistry*, **6**, 3458–3464.

REGOLI, D. & VANE, J. R. (1964). A sensitive method for the assay of angiotensin. *Br. J. Pharmac. Chemother.*, **23**, 351–359.

Inhibition of prostaglandin synthesis augments the effects of sympathetic nerve stimulation on the cat spleen

S. H. FERREIRA and S. MONCADA*

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

Indomethacin blocks the synthesis of prostaglandins E_2 and $F_{2\alpha}$ from arachidonic acid by cell-free homogenates of guinea-pig lungs (Vane, 1971) and the release of prostaglandins from dog spleens when they are contracted by adrenaline (Ferreira, Moncada & Vane, 1971). Hedqvist (1970) proposed that prostaglandin release by the spleen is a feed-back mechanism controlling the output of noradrenaline from sympathetic nerves. The following results support this hypothesis.

Nine cats were anaesthetized with pentobarbitone sodium (30 mg/kg i.m.). The splenic pedicle was dissected to separate artery, vein and nerves. The spleen was removed and perfused with Krebs solution containing dextran (2–3% w/v) at 8–15 ml/minute. Part of the splenic outflow superfused assay tissues which detected prostaglandins (Ferreira, Moncada & Vane, 1971). Perfusion pressure and spleen weight were measured.

The resting spleen perfusion pressure and weight remained stable for several hours. Stimulation of the splenic nerve (1–3 ms pulse, 5–30 V, 1–10 pulses/s, trains of 20–120 s) gave reproducible rises in perfusion pressure and falls in spleen weight. There was also an output of prostaglandin into the perfusate which, when measured as E_2, represented an increase of 1–20 ng/ml.

When indomethacin (0·3–5 μg/ml) was infused into the spleen there was no change in spleen weight but the perfusion pressure increased, sometimes by as much as 250%. At the same time, there was a decrease in the basal secretion of prostaglandins, as shown by relaxation of the assay tissues. When nerve stimulation was repeated, the contraction of the spleen was greater than before. The rise in perfusion pressure