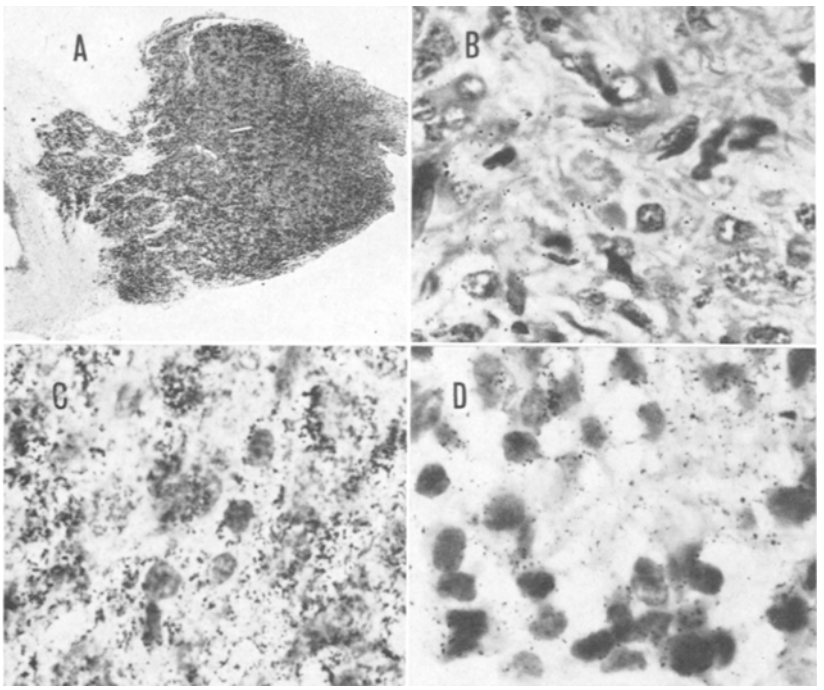

(A) Autoradiogram of section of pineal gland and adjacent brain tissue from cynomolgus monkey injected 25 mCi ^{3}H-5-HTP 60 min after administration of MAO inhibitor. It shows dense silver grain deposition precisely localized over gland tissue indicating presence of high concentration of isotope. Nuclear fast red and tartrazin (× 30). (B) Autoradiogram of section of pineal gland from cynomolgus monkey injected 25 mCi ^{3}H-5-HTP with no MAO inhibitor showing sparse silver grain deposition. Compare with (C) and (D). Nuclear fast red and tartrazin (× 350). (C) and (D) Autoradiograms of section of pineal gland from the same monkey as shown in (A) showing silver grain distribution concentrated mainly over cytoplasm and cytoplasmic processes. Nuclear fast red and tartrazin (× 300).

Grain counts per pinealocyte were 5 times greater in this animal than in the former and grain distribution was concentrated over the cytoplasm and cytoplasmic processes of the cell. There was minimal labelling over the nucleus (Figure D). In the autoradiographs prepared from the uninjected animal no evidence of chemical fogging of the emulsion was found.

Silver grain deposition over pinealocytes shows that these structures contain isotope. In the present study the sparseness of grains in the gland of the monkey not given MAO inhibitor indicates a low tissue concentration of isotope and implies that isotope is being removed. However, removal of any isotope from these cells by MAO depends on the conversion of ^{3}H-5-HTP to ^{3}H-5-HT by the enzyme aromatic L-amino acid decarboxylase[7] because ^{3}H-5-HTP is not a substrate for MAO[8]. Since the pineal gland contains the enzymes necessary to synthesize and destroy 5-HT[3,7], the low concentration of isotope in this animal is very likely due to the uninhibited breakdown of synthesized ^{3}H-5-HT by MAO. This hypothesis is supported by the demonstration of a high concentration of isotope in the animal in which MAO activity was inhibited. These findings are similar to those made earlier[9] on rat brain neurones in vivo and in tissue culture and are consistent with the known rapid turnover of 5-HT[10–12].

Zusammenfassung. Die Verteilung von aus 5-Hydroxy-Tryptophan entstandenem Serotonin in der Zirbeldrüse von Affen wird beschrieben.

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Comparison of β-Adrenergic Blocking Activity of DCI, H56/28, ICI50172, LB46, Methoxamine, MJ1999 and Propranolol in the Blood Perfused Canine Papillary Muscle Preparation

Since β-adrenergic blocking action of DCI[1] was found, many active compounds have been successively synthesized in the past decade. Qualitative differences, however, were found among different β-adrenergic receptors[2]. Previously we compared the relative potency of DCI, methoxamine, propranolol, MJ1999, H56/28, LB46 and ICI 50172 to block the positive chronotropic effect of isoproterenol which was given intra-arterially into the sinus node artery[3]. In this study we compared the potencies of these compounds to block the positive inotropic effect of norepinephrine using the blood perfused papillary muscle preparation of dogs.

The heart was removed from a dog, anesthetized with ether and plunged into the cold Tyrode's solution. The

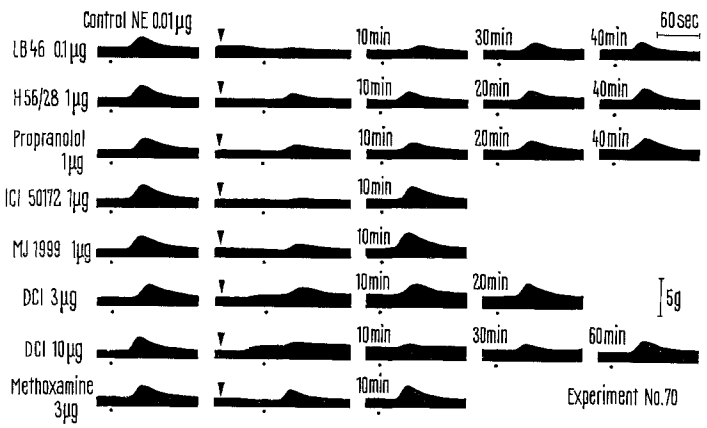

Fig. 1. Comparison of 7 β-adrenergic blocking compounds in the blood perfused papillary muscle preparation. Triangles indicate the time when test compound (antagonist) was administered and dots indicate that for injection of norepinephrine (agonist). Experiment was done in the same preparation (Experiment No. 70).

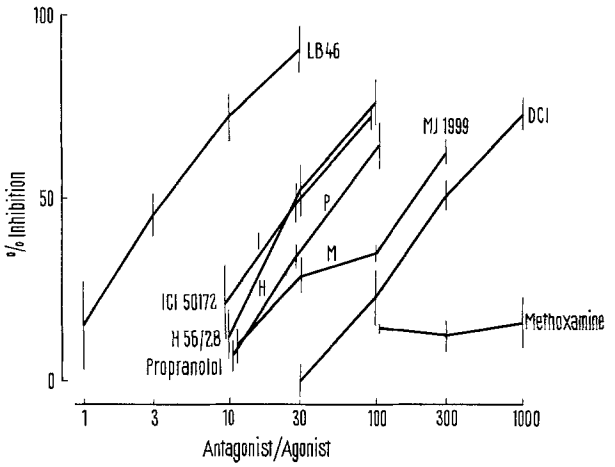

Fig. 2. Relative potencies of 7 β-adrenergic blocking compounds. Abscissa: antagonist-agonist ratio in logarithmic scale; and ordinate: percent inhibition.

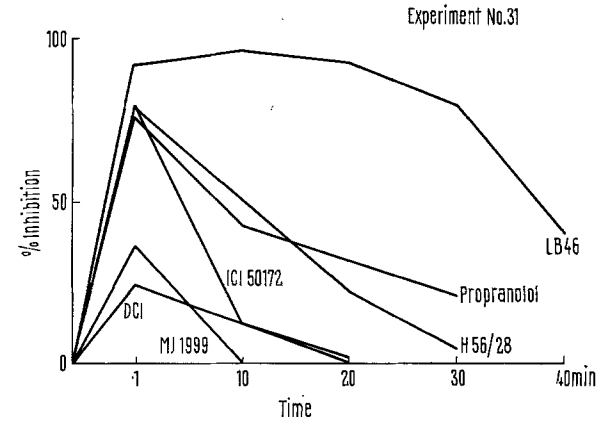

Fig. 3. Duration of β-adrenergic blocking action of 1 µg of 6 β-adrenergic blocking compounds.

anterior septal artery was dissected and cannulated. All branches except the arteries to the anterior papillary muscle were ligated. The isolated muscle was perfused with the arterial blood of a donor dog by the aid of a Sigmamotor pump. A pneumatic resistance was placed in parallel with the perfusion system so that a constant perfusion pressure at 100 mm Hg was maintained. Blood was warmed to 38–39 °C. The tendinous end of the papillary muscle was connected to a force displacement transducer (Grass FT 03 B) by a fine silk thread and stretched with a weight of 0.5–1.5 g. The papillary muscle was electrically driven at a frequency of 120 beats/min by an electronic stimulator (1–2 V, 5 msec and 2 cps). The details of the experimental set-up were described in the previous papers[4,5].

Norepinephrine was used as an agonist because the positive inotropic response of the papillary muscle was well observed by use of norepinephrine rather than epinephrine[6]. The dose of 0.01 µg of norepinephrine given into the perfusion system caused almost the same response throughout the experiment over 10 h. Thus 1 papillary muscle preparation was sufficient enough to test all 7 compounds. A typical result is shown in Figure 1 (Experiment No. 70). Different doses of an antagonist were given and 0.01–0.03 µg of norepinephrine was challenged 1, 10, 20, 30, 40 and 60 min after administration of an antagonist. DCI caused the apparent and long-lasting positive inotropic effect, while LB46, MJ 1999, propranolol and ICI 50172 depressed slightly the contractile force of the papillary muscle. H 56/28 and methoxamine had no significant depressive effect except with large doses.

The potencies to block the β-adrenergic effect of norepinephrine are shown in Figure 2. Relative potencies are as follows: LB46 > ICI 50172 = H 56/28 = propranolol > MJ 1999 > DCI > methoxamine which roughly corresponds to 1, 1/10, 1/30, 1/100 and less than 1/100. Methoxamine did not show any dose-response relation. The duration of blocking effect of 1 µg of each compound is shown in Figure 3 (Experiment No. 31). The duration of blocking effect is over 40 min with LB46, about 30 min with propranolol or H 56/28 and about 10 min with DCI, ICI 50172 and MJ 1999. The positive inotropic effect induced by 0.3 mg of calcium chloride was not modified by any β-adrenergic blocking compound, the dose of which sufficiently blocked 0.01–0.03 µg of norepinephrine.

Comparing blocking potencies of these compounds between the positive chronotropic and inotropic effects of catecholamines in dogs, LB46 shows the most potent β-adrenergic blocking activity against both effects. LB46,

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ICI 50172, MJ 1999 and methoxamine show almost similar β-adrenergic blocking potencies either in the papillary muscle contraction or in the sinoatrial pacemaker activity, while propranolol, H 56/28 and DCI show more potent blocking activity on the chronotropic effect of catecholamine than on the inotropic one.

Zusammenfassung. Nachweis, dass der β-Blocker LB 46 am blutperfundierten Papillarmuskel des Hundes eine grössere Aktivität aufweist als 6 andere bekannte β-Blocker.

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Specific Oedema-Inhibiting Property of a Natural Anti-inflammatory Factor Collected from Inflamed Tissue

While studying the effect of a series of copper-containing compounds (e.g. $Cu(OH)_2CuCO_3$) we found that the anti-inflammatory activity of most of them was regularly associated with marked tissue irritation at the site of injection, and that those compounds which did not provoke irritation were devoid of an anti-inflammatory effect[1]. We postulated that tissue irritation by the copper compounds may have released into the circulation a substance which inhibits inflammation at other sites in the organism. Several investigators[2–8] have noted that the administration of irritant substances inhibited experimental inflammation, but little is known about the specificity of this natural anti-inflammatory factor. In this communication we report the effects of a natural anti-inflammatory factor on different types of oedemas, which differ from each other in their mechanism and also in their responses to drugs[9,10].

Materials and methods. Male albino rats of a strain bred at TNO Animal Centre (Netherlands) were used throughout. Local tissue irritation was induced by i.p. injection of phenylquinone (0.03% solution, 1 ml/rat). Paw oedema was induced and measured according to methods described earlier[9]. Inflammatory pouches were produced by the method of Boris and Stevenson[11] and the exudate removed 4 days following the induction of the pouch. On average 1 animal yielded 9–10 ml of exudate which was then dialyzed and lyophilized. Gel filtration was carried out on Sephadex G-75, G-100 and G-150 columns (2.5 × 50 cm). Equilibration of the gel and elution were performed with 0.1 *N* acetic acid (pH 3.2).

Results and discussion. Table I shows that in rats which received an i.p. injection of phenylquinone, there was a marked inhibition of paw swelling by kaolin but not by serotonin or polyvinyl-pyrrolidone (PVP). These results indicated that tissue irritation caused by phenylquinone exerts a remote anti-inflammatory effect specifically towards those particular type of oedemas which are also inhibited by phenylbutazone.

In an attempt to transfer the postulated anti-inflammatory tissue factor into other rats, we demonstrated earlier that peritoneal exudate and paw oedema fluid collected from rats, inhibited the kaolin-induced paw swelling when injected into other rats[12]. In the present investigation we have examined the effect of exudates collected from inflammatory pouches. The water-soluble component of the exudate was found to contain the factor which inhibited the kaolin-induced rat paw oedema, and dialysis of the water-soluble portion revealed that the activity is due to a high molecular weight material, as most of the activity was found in the retentate. As seen in Table II, about 100 mg/kg of lyophilized retentate reduced the swelling of the rat paw to 50% when injected i.p., whereas 500 mg/kg dialysate reduces to only 32%.

We have further examined the activity of the water-soluble, non-dialyzable principles of the pouch material against the inflammation caused by kaolin, carrageenin, serotonin, histamine and PVP. We found that serotonin, histamine and PVP oedemas are not inhibited, whereas kaolin and carrageenin oedemas are definitely inhibited by this anti-inflammatory factor present in the pouch material (Table III). Inhibition of the kaolin-induced oedema was more pronounced than that of carrageenin oedema. We have regularly observed, however, that with our rat strain the carrageenin oedema is more resistant to inhibitory effects than the kaolin oedema. Previous work has shown that phenylbutazone counteracts the oedema

Table I. Hind-paw oedema. nhibition in peritoneally irritated and phenylbutazone treated rats

Pretreatment	Inhibition (%)		
	Kaolin[a]	Serotonin[a]	PVP[a]
Phenylquinone, i.p. irritation	49 (41–57)[b]	0	0
Phenylbutazone, orally 100 mg/kg	83 (74–92)	0	0

[a] Oedema inducer. [b] Figures are mean values of groups of 10 rats each. In brackets: 95% fiducial limits.

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