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EFFECTS OF (+)- AND (−)-PROPRANOLOL ON THE RESPONSES OF THE HUMAN ISOLATED BASILAR ARTERY TO CEREBROSPINAL FLUID OBTAINED FROM PATIENTS WITH SUBARACHNOID HAEMORRHAGE AND CEREBRAL ARTERIAL SPASM

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1 The human isolated basilar artery has been used as a model to investigate the aetiology of cerebral arterial spasm associated with rupture of intracranial aneurysms.

2 The isolated artery is contracted by 5-hydroxytryptamine, noradrenaline, six prostaglandins and cerebrospinal fluid from patients with ruptured aneurysms and cerebral arterial spasm.

3 These contractions are reversed by (±)−, (+)− and (−)-propranolol in concentrations known to produce local anaesthetic effects on isolated frog sciatic nerve; the (+) isomer was 2.5 to 10 times more potent than (−)-propranolol in antagonising all contractions.

4 As the two isomers are known to have similar local anaesthetic potency but (−)-propranolol has greater β-adrenoceptor blocking effects we conclude that the antagonistic effects described do not involve β-adrenoceptor blockade.

5 The data indicate that propranolol may be of clinical use in reversing cerebral arterial spasm.

Introduction

Many investigators believe that various pharmacologically active agents including 5-hydroxytryptamine (5-HT), prostaglandins, catecholamines and unidentified substances are responsible for the prolonged cerebral arterial spasm which is a salient feature of subarachnoid haemorrhage (SAH) which follows rupture of cerebral aneurysms and is believed to be the cause of considerable mortality and morbidity (Robertson, 1974).

We favour the view that this arterial spasm has, in the main, a pharmacological basis and that there are two feasible approaches to the problem of the reversal of spasm in patients. The first may be termed the specific approach: it requires the isolation and identification of the vasoactive substances responsible for spasm and the synthesis of specific pharmacological antagonists.

We are currently investigating this problem by a variety of ways, including studying the responses of the isolated human basilar artery to drugs and cerebrospinal fluid (CSF) (Starling, Boullin, Grahame-Smith, Adams & Gye, 1975). This arterial preparation contracts to CSF from SAH patients and there appears to be an unidentified contractile material present in the CSF of approximately 66% of SAH patients with preoperative spasm but only 16% of patients without overt angiographic evidence of spasm (Boullin, Mohan & Grahame-Smith, 1976).

We have also utilised a second, non-specific approach using the basilar artery model by searching for drugs which non-selectively reverse the contractions induced by CSF from patients with subarachnoid haemorrhage.

Any drugs which are potent in reversing CSF contractions *in vitro* may become candidates for clinical use; propranolol seems to be such a drug.

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Methods

Subjects

The subjects were eight patients aged 49-64 years who presented at the Radcliffe Infirmary, Oxford, with subarachnoid haemorrhage from ruptured cerebral aneurysms. Clinical details are presented elsewhere (Boullin, *et al.* 1976). They underwent carotid and/or vertebral angiography to determine the site of aneurysm and the presence or absence of cerebral arterial spasm. Lumbar CSF was obtained from these patients 2-10 h after angiography. Control CSF was obtained from twelve patients (three females, nine males, aged 19-53 years) undergoing myelography for suspected prolapsed intervertebral disc.

Collection and preparation of CSF

After collection, the CSF was immediately cooled to 4° C and centrifuged at 3000 *g* for 5 min to remove cells and debris. The clear supernatant was frozen at −20° C, lyophilised at −80° C and then the dried material was stored at −20° C.

Isolated basilar artery

The setting up of the human isolated basilar artery has been described in detail in an earlier paper (Starling *et al.*, 1975). The preparation was set up in a 13 ml isolated organ bath, in Krebs solution gassed with 5% CO_2 in O_2 and maintained at 37° C. The muscle was cut spirally rather than by making alternate transverse cuts; this did not affect the arterial responses to drugs. The muscle was attached to an isotonic transducer (Model 1326, C.F. Palmer, High Wycombe, Bucks) coupled to a Tekman TE200 Single Channel Potentiometric Recorder (Tekman Electronics Ltd, Bicester, Oxon) and a weight of 0.75 g was applied.

Arterial responses to CSF and drugs

Drugs were dissolved in Krebs solution and applied to the artery in a 13 ml organ bath in volumes not exceeding 200 μl. Addition of these volumes of Krebs solution did not change arterial responses.

Aliquots of lyophilised CSF were reconstituted in distilled water and added to the organ bath in volumes of 50-500 μl. These volumes of normal CSF did not affect arterial responses (Starling *et al.*, 1975).

Drugs

5-HT and noradrenaline (NA) were obtained from Sigma Chemical Company, Kingston, Surrey. The following prostaglandins were kindly donated by Dr J.E. Pike, The Upjohn Company, Kalamazoo, MI 49001, USA: E_1 (PGE_1); E_2 (PGE_2); A_1 (PGA_1); A_2 (PGA_2); $F_{1\alpha}$ ($PGF_{1\alpha}$). $F_{2\alpha}$–tromethamine salt ($PGF_{2\alpha}$). ±-propranolol and isomers were donated by ICI Ltd, Macclesfield, Cheshire.

All drug concentrations are expressed in terms of molarities of the base and represent the concentrations of drug present in the organ bath.

Results

Effects of propranolol on CSF induced contractions

As described recently, CSF from patients presenting with SAH and angiographic evidence of cerebral arterial spasm contracts the isolated human basilar artery in a majority of cases (Boullin, Grahame-Smith, Mohan & Starling, 1975a; Boullin, Starling, Grahame-Smith & Mohan, 1975-6; Boullin *et al.*, 1976).

Tests with CSF from three patients with SAH and cerebral arterial spasm showed that 20 μmol/l (±)-propranolol produced a rapidly developing and sustained relaxation of the artery which was maximal after 35 min. A further application produced additional relaxing effects again taking 35 min to reach a maximum (Figure 1). Subsequently we investigated the effects of the separate isomers of propranolol in five experiments.

As shown in Figure 2 high concentrations (100 μmol/l) of either isomer of propranolol reverses the contractions, causing rapid relaxations of the arterial muscle. The rate of relaxation with (+)-propranolol was 7.8 cm/min which was 5.6 times faster than with (−)-propranolol (1.4 cm/min). A similar difference was observed regarding the threshold concentrations of (+)- or (−)-propranolol producing relaxation. Thus Figure 3 shows that 15 μmol/l (−)-propranolol had no effect upon a CSF induced basilar artery contraction, whereas the same concentration of the (+)-isomer produced a relaxation at a rate of 0.9 cm/min. The threshold concentration of (+)- or (−)-propranolol required to antagonise CSF induced contractions showed considerable variation, which may reflect the fact that the arteries

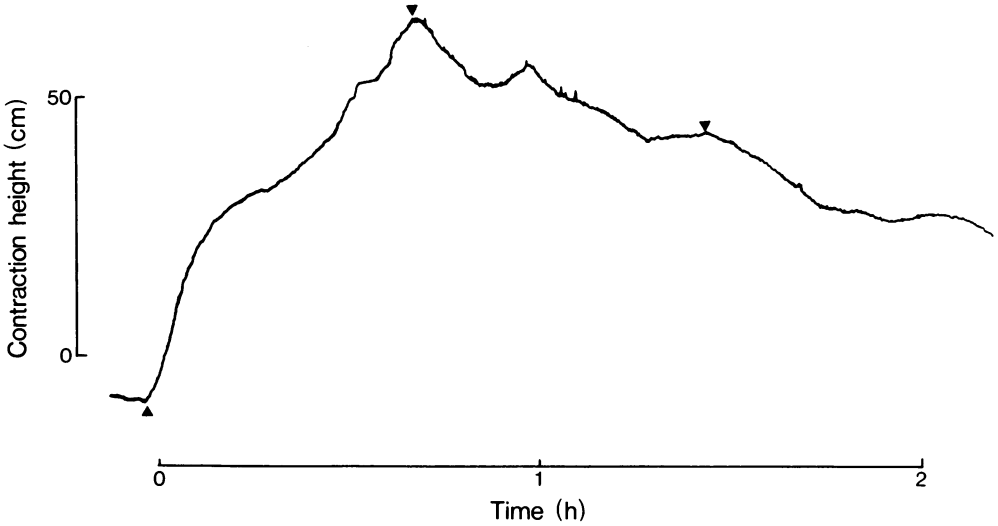

Figure 1 Reversal of CSF-induced contraction of the human isolated basilar artery by (±)-propranolol. The responses of the basilar artery to lyophilised CSF (200 μl) reconstituted in distilled water from a patient with a subarachnoid haemorrhage with preoperative arterial spasm (see **Methods**) are shown. CSF was added at ▲. After about 45 min 20 μmol/l (±)-propranolol was added (▼) and the muscle relaxed; a second dose (20 μmol/l) (▼) added 45 min thereafter produced a further relaxation.

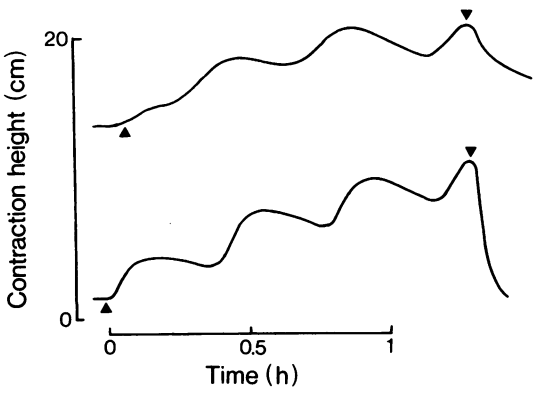

Figure 2 Reversal of CSF induced contractions of the human isolated basilar artery by (+) and (−)-propranolol. Responses of a basilar artery to lyophilised CSF (200 μl) reconstituted in distilled water (see **Methods**) added at ▲ are shown. Upper record, reversal of CSF induced contraction by 100 μmol/l (−)-propranolol (▼); lower record, similar but greater relaxation induced by 100 μmols/l (+)-propranolol (▼). In each case the relaxations produced by (+) and (−)-propranolol continued for longer times than illustrated, and rhythmic activity was not resumed.

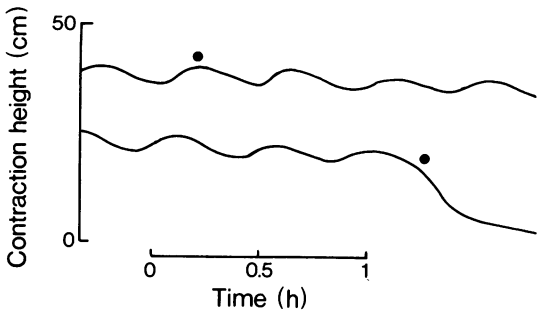

Figure 3 Differential effect of (+) and (−)-propranolol in reversing CSF induced contractions of the human isolated basilar artery. The basilar artery was contracted with CSF (200μl) as described in Figure 1 and the responses illustrated were obtained 60 min thereafter. Upper record: lack of effect of 15 μmol/l (−)-propranolol (●): lower record: prolonged relaxations induced by 15 μmol/l (+)-propranolol (●).
The data are presented in the same form as Figure 1.

were obtained 1-4 days after death (Starling *et al.*, 1975). In five experiments we observed that the concentration of (+)-propranolol required to relax the artery was 2.5-10 times lower than the concentration of the (−)-isomer. The range of threshold concentrations of the (−)-isomer was 50-100 μmol/l and 10-20 μmol/l for the (+)-isomer (Table 1).

As described above, the relaxations produced by (+)-propranolol were more rapid and quantitatively greater than those produced by (−)-propranolol. On the other hand, the relaxations produced by both isomers persisted for the duration of contact with the artery, although after washing, further reproducible contractile responses to CSF could be obtained.

Table 1 Reversal of CSF-induced contractions of the basilar artery by propranolol

Subject	Age (years)	Sex	Threshold concentration of propranolol (μmol/l) reversing CSF-induced contractions (±)	(+)-isomer	(−)-isomer
1	48	F	20	–	–
2	64	F	20	–	–
3	46	F	–	15	50
4	55	F	–	10	50
5	56	F	–	10	100
6	49	M	20	–	–
7	54	M	–	15	100
8	49	M	–	20	50

Effects of propranolol on contractions produced by other drugs

The above results raised the question of the specificity of the propranolol induced antagonism in regard to β-adrenergic receptor blockade.

The following evidence supports the view that the propranolol induced relaxations were not related to selective blockade of β-adrenergic receptors.

Earlier experiments showed that 5-HT, noradrenaline and six prostaglandins contract the isolated basilar artery (Boullin *et al.*, 1975a; Boullin *et al.*, 1975b; Starling *et al.*, 1975) although the time course of the contractions is different from those produced by CSF (Boullin *et al.*, 1976). These observations were repeated and we found that propranolol reversed contractions induced by all of these agents, and moreover relaxed arteries which displayed spontaneous contractile activity. In each case, (+)-propranolol was at least three times more potent than (−)-propranolol in antagonising the contractions.

Discussion

Our results strongly suggest that propranolol relaxes the human isolated basilar artery by some non-specific mechanism unrelated to blockade of β-adrenergic receptors. The drug antagonises contractions produced by a variety of compounds which may be involved in the aetiology of cerebral arterial spasm in addition to CSF from SAH patients with spasm and also contractions occurring spontaneously.

Previously Rosenblum (1969) showed that propranolol antagonised pial arterial spasm in animals, but again this was not related to β-adrenoceptor blockade. Moreover as lignocaine did not reverse the spasm, the local anaesthetic properties of propranolol may not have been involved in this antagonism.

There is a dose-response differential between the (+)- and (−)-isomers of propranolol in our experiments with the greater potency being shown by the (+)-isomer. This difference in favour of the (+)-isomer which has only one fortieth to one hundredth of the β-adrenoceptor blocking action of (−)-propranolol (Barrett & Cullum, 1968; Fitzgerald, 1969; Dollery, Paterson & Conolly, 1969) suggests the relaxation is also not due to β-adrenoceptor blockade. However, as the isomers have approximately equal local anaesthetic activity (Barrett & Cullum, 1968) we believe that the action of propranolol in relaxing the artery is due to some quinidine-like local anaesthetic action plus some other unidentified pharmacological component which predominates with (+)-propranolol; lignocaine had similar effects to propranolol on the basilar artery.

The question arises as to whether these *in vitro* observations can be extrapolated to the clinical situation in regard to treatment of cerebral arterial spasm associated with SAH.

There is a 20-fold variation in plasma propranolol concentrations following oral administration (Shand, 1974) but it is generally accepted that values of 50-100 ng/ml (193-386 nmol/l) are required for significant cardiovascular β-adrenoceptor blocking actions and that these concentrations are obtained with doses of 40-80 mg three times daily (Shand, 1974). We found that in most experiments 20 μmol/l (±)-propranolol effectively antagonised spontaneous contractions, contractions induced by CSF, 5-HT, prostaglandins and noradrenaline. This is clearly a much higher concentration than that found clinically. In some instances 2 μmol/l was effective *in vitro* and much higher doses of propranolol, up to 3 g daily, have recently been tested in schizophrenia (Yorkston, Zaki, Malik, Morrison & Havard, 1974) where

presumably much higher plasma concentrations are attained. On this basis it is plausible that propranolol might be tested clinically to see if there is a reversal of arterial spasm following SAH. Du Boulay, Symon, Kendal & Crockard (1974) have shown that intracarotid propranolol dilates spastic cerebral arteries following experimental SAH in baboons.

On the other hand, intracarotid injection of propranolol (2 mg) produced a *reduction* in cerebral blood flow and oxygen consumption accompanied by an increase in cerebrovascular resistance in fourteen patients with cerebral infarction (Meyer, Okamoto, Sari, Koto, Itoh & Ericsson, 1974). Although the changes observed were rather small (8-11% decrease in cerebral blood flow), the problems associated with systemic administration of drugs may be circumvented by giving propranolol, and possibly other compounds with similar actions on the basilar artery such as lignocaine, directly into the subarachnoid space surrounding the circle of Willis at operation and subsequently via a catheter into that space; this technique is currently being evaluated at the Radcliffe Infirmary.

We wish to thank Mr C.B.T. Adams for allowing us to study his patients.

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