Arterial catecholamine and enzyme histochemistry in migraine

C. W. M. ADAMS, C. C. ORTON, AND K. J. ZILKHA

From the Department of Pathology, Guy's Hospital Medical School, Department of Neurology, King's College Hospital, and The National Hospital, Queen Square, London

The purpose of this communication is to present details of a histochemical investigation of superficial temporal arteries biopsied during an acute attack of migraine. Particular emphasis has been directed towards the demonstration of catecholamines, monoamine oxidase, and cholinesterase activity in the vessels, because disturbed local synthesis or discharge of these agents might reasonably be considered as a possible precipitating factor in migraine.

METHODS

Biopsies of dilated superficial temporal arteries were obtained under local anaesthesia from six volunteer subjects during a severe attack of migraine and within a few hours from the onset of the attack. They had all suffered from recurrent severe migraine over many years (Table I) and had complained of localized pain and tenderness over a segment of the superficial temporal artery during attacks. These patients usually obtained relief with 2 mg ergotamine tartrate taken either orally or by rectal suppository soon after the onset of the attack but, on this occasion, they had delayed such treatment until after the biopsy. Control biopsies were obtained at craniotomy from six non-migrainous subjects undergoing neurosurgical procedures. These patients were aged from 38 to 59 yr. All subjects were normotensive.

TABLE I

CLINICAL DETAILS OF PATIENTS WITH MIGRAINE

Sex	Age	Length of History of migraine (yr)	Usual Therapy
E.T.	F	33	10
B.P.	F	40	15
H.R.	M	56	40
B.R.	F	43	11
R.S.	M	58	55
J.B.	F	55	28

The biopsied arteries were transported to the laboratory over ice in a Thermos flask. Part of each artery was fixed in 4% formaldehyde-saline at 4°C, while the other

part was rapidly frozen onto a cryostat chuck by means of a carbon dioxide jet. Conventional cryostat sections were used for dehydrogenase, phosphatase, and monoamine oxidase methods. For catecholamine studies, cryostat sections were either dehydrated within the cryostat (that is, freeze-dried) for 40 min or desiccated over solid carbon dioxide under reduced pressure (Hamberger and Norberg, 1964); the former method was found to be the most satisfactory in practice. The sections were warmed with the operator's hand before removing them from the cryostat; this manoeuvre prevented condensation of water over them on exposure to laboratory atmosphere.

Cholinesterase activity was demonstrated in cryostat sections of tissue that had been fixed in cold formalin for 18 hr. The rest of the formalin-fixed material was embedded in paraffin for conventional microscopy.

Lactic dehydrogenase, reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH₂-TR) and adenosine triphosphatase (ATPase) activities were demonstrated by methods previously described by Adams, Davison, and Gregson (1963). Monoamine oxidase activity was displayed by Glenner, Burtner, and Brown's (1957) method, with tryamine as substrate and nitroblue tetrazolium as the electron acceptor. Cholinesterase activities were demonstrated by Karnovsky and Roots' (1964) technique, with acetylthiocholine and butyrylthiocholine as substrates. The use of specific inhibitors of true and pseudocholinesterases proved to be unnecessary (see Results).

Catecholamines were detected by the ultraviolet fluorescence of their isoquinoline derivatives after exposure to paraformaldehyde vapour at about 70% relative humidity in a closed container at 80°C for 2 hr (Falck, 1962; Falck, Hillarp, Thieme, and Torp, 1962). For ultraviolet fluorescence microscopy, Leitz BG12 and Blau BG38 filters were used for excitation and a Leitz K530 mμ filter was inserted as an ultraviolet barrier above the objective lens. The specificity of paraformaldehyde-induced fluorescence for catecholamines was tested by reducing the resulting isoquinolines to their non-fluorescent tetra-hydro derivatives with 0.1 sodium borohydride in 95% ethanol (Corrodi, Hillarp, and Jonsson, 1964). The catecholamine-binding capacity of adrenergic nerve fibres was assessed after incubating cryostat sections in aqueous noradrenaline (1 mg/ml.) for 45 min (Angelakos and King, 1965). Some sections

were preincubated in aqueous desipramine (Pertofran; 3 mg/ml.) for 15 min, before incubating for 45 min in a mixture of *noradrenaline* (1 mg/ml.) and desipramine (3 mg/ml.) (Carlsson, Fuxe, Hamberger, and Lindqvist, 1966). In order to determine the effect of ergotamine tartrate on periarterial adrenergic nerve fibres, some sections were incubated for 45 min in either ergotamine tartrate (0.05 mg/ml.) or ergotamine tartrate-*noradrenaline* mixture (0.05 mg/1 mg/ml.). Further to test this last point, a rat was injected subcutaneously with 0.03 mg of this drug and was then killed 1 hr later. The aorta from this rat and from an untreated animal were sectioned and examined for catecholamine fluorescence.

Selected fresh or formalin-fixed paraffin-embedded tissues were stained with haematoxylin and eosin, Verhoeff's elastic, Van Gieson, and Glees and Marsland's silver stains.

RESULTS

MORPHOLOGY No abnormalities are apparent in paraffin sections of migrainous temporal arteries. No inflammatory changes, oedema, thickening or proliferation are seen in the tunica media, while the medial muscle fibres and elastic laminae appear normal. The tunica adventitia is essentially normal, but may be slightly thickened with collagen (see Torda and Wolff, 1945; Wolff, 1963). The tunica intima is often diffusely thickened by collagen, smooth-muscle and elastic fibres. However, such intimal thickening is within the normal range for temporal arteries in normotensive subjects of the same age as the migraine patients (Ainsworth, Gresham, and Balmforth, 1961; Missen, 1963).

ENZYMES Lactic dehydrogenase, NADH₂-TR, and ATPase activities are not altered in migrainous arteries. Cholinesterase is equally active in ill-defined, very scanty adventitial nerve fibres of both control and migrainous arteries. Monoamine oxidase activity in medial smooth muscle fibres is somewhat variable; ranking the intensity of reaction shows no clear difference between control and migrainous vessels (see Table II; Figs. 1 and 2).

TABLE II

HISTOCHEMICAL MONOAMINE OXIDASE ACTIVITY IN TEMPORAL ARTERIES RANKED ACCORDING TO INTENSITY OF REACTION

Migraine patients		Control subjects	
M1	2	C1	—
M2	3	C2	6
M3	9	C3	1
M4	8	C4	5
M5	7	C5	4
M6	11	C6	10
mean rank	6.7	mean rank	5.2

(1 = strongest, 11 = weakest)

CATECHOLAMINES A number of fibres are revealed in the tunica adventitia with the paraformaldehyde fluorescence technique (Figs. 3 and 6). Such fibres disappear after immersing the section in either water or alcoholic borohydride (Fig. 4). After treatment with the latter reagent the fibres reappear when fluorescence is regenerated with paraformaldehyde. However, we were doubtful about the true nature of these fibres. Thus, they are refractile in ordinary transmitted light (Fig. 5) and frequently appear as parallel lines, neither of which features are characteristic of nerve terminals. These fibres could not be convincingly stained by either Glees and Marsland's axon stain or by Verhoeff's elastic method. Thus, the possibility arose that they may be the curled edges of dried collagen fibres. The apparent fluorescence of these refractile fibres is in fact artefactual; unlike the diffuse fluorescence of the main mass of adventitial collagen (see below), such artefactual fluorescence is extinguished when the section is mounted in liquid paraffin (Fig. 7). The extinction of artefactual 'fluorescence' engendered by immersion in water or alcoholic borohydride (Fig. 4) is perhaps due to flattening of collagen fibres after the section has been moistened. This explanation is strengthened by the observation that artefactual fluorescence reappears when the borohydride- or water-treated section is air-dried for some 30 min. The reappearance of 'fluorescence' after further treatment with hot paraformaldehyde can thus be ascribed to the effects of drying.

COMMENT ON FLUORESCENCE ARTEFACTS It is clear that artefactual fluorescence is encountered when air-dried unmounted sections are viewed in ultra-violet light. If sections are to be subsequently treated with a blocking agent or stained for enzyme activity, it is obviously impractical to mount them in liquid paraffin or other non-fluorescent medium. Under these circumstances great care is needed to distinguish artefactual from real fluorescence. It should also be noted that arterial elastic laminae are fluorescent without exposure to paraformaldehyde, but such autofluorescence is unlikely to cause difficulty in interpretation.

CATECHOLAMINE-BINDING IN MIGRAINOUS ARTERIES Although the above-mentioned scattered 'fluorescent' fibres in the tunica adventitia are artefactual (Figs. 3 and 6), the main mass of adventitial collagen around migrainous arteries shows a marked capacity to absorb *noradrenaline* after incubating in this catecholamine. The diffuse fluorescence of the adventitia illustrated in Figs. 6-8 is characteristic of all six migrainous arteries, whereas the relative absence of such fluorescence—as shown in

Symbols in the figures: IEM = internal elastic membrane, AMJ = adventitio-medial junction. In all illustrations the tunica adventitia is at the top and the tunica intima at the bottom.

FIG. 1. Monoamine oxidase activity in migrainous temporal artery. Tyramine-nitroblue tetrazolium, $\times 780$.

FIG. 2. As for Fig. 1 in control temporal artery.

FIG. 3. Trivial noradrenaline-binding in tunica adventitia of control temporal artery. Note artefactually fluorescent fibre (arrow). Incubated in noradrenaline, Falck's paraformaldehyde fluorescence method $\times 780$.

FIG. 4. Same artery as Fig. 3 but after inhibition of fluorescence by borohydride. Note that artefactual fibres have disappeared.

FIG. 5. Artefactual refractile fibre (arrow) at adventitia-medial junction. Same field as Fig. 6, but photographed in ordinary transmitted light $\times 780$.

FIG. 6. Strong noradrenaline-binding in tunica adventitia of migrainous temporal artery. Note artefactually fluorescent fibre (arrow) corresponding to that shown in Fig. 5. Incubated in noradrenaline, paraformaldehyde fluorescence method $\times 780$.

FIG. 7. Same field as Fig. 6, but mounted in liquid paraffin to show disappearance of artefactual fibre but persistence of diffuse fluorescence in tunica adventitia.

FIG. 8. Strong noradrenaline-binding in tunica adventitia of another migrainous temporal artery. Incubated in noradrenaline, paraformaldehyde fluorescence method $\times 780$.

Fig. 3—is typical of all six control vessels. Such fluorescence is entirely inhibited by preliminary treatment with borohydride (Fig. 9) and can be partly regenerated by re-exposure to paraformaldehyde vapour (Fig. 10); it does not disappear when the section is mounted in liquid paraffin (Fig. 7). It is irreversibly inhibited by immersion in water and does not reappear when the section is air-dried.

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FIG. 9. Same tissue as Figs. 6 and 7 to show loss of paraformaldehyde induced fluorescence in tunica adventitia after treatment with borohydride $\times 960$.

FIG. 10. Same tissue as Fig. 9, but after regeneration of specific fluorescence by re-exposure to paraformaldehyde.

Fluorescence is not altered by incubation of the artery in the desipramine-*noradrenaline* and ergotamine-*noradrenaline* mixtures. No paraformaldehyde-induced fluorescence is seen in the adventitia of either migrainous or control temporal arteries, when sections are not incubated in *noradrenaline*—that is, no *endogenous* catecholamines were detected with our technique in human temporal arteries. Incubation in ergotamine does not induce fluorescence.

In one additional case of migraine (and a corresponding control), we tested the effect of incubating sections in serotonin creatinine sulphate (2 mg/ml.): no convincing induced fluorescence is seen in either control or migrainous arteries.

CATECHOLAMINES IN RAT AORTA Fluorescent adrenergic fibres are seen in the adventitia and outer media of the rat aorta. Fluorescence is increased in sections incubated in *noradrenaline*, but some endogenous catecholamine is apparent in these fibres in unincubated tissue. Such fluorescence persists after the section is mounted in liquid paraffin, is extinguished by borohydride and is partially regenerated by re-exposure to paraformaldehyde. Injection of ergotamine into the rat does not alter the fluorescence in these aortic adventitial nerve fibres. However, the intensity of fluorescence is reduced in aortic sections incubated in a desipramine-*noradrenaline* mixture compared with those incubated in *noradrenaline* alone.

DISCUSSION

Histological examination of migrainous temporal arteries, excised during an acute attack, reveals no convincing structural abnormality in any of the vascular coats. Histochemical studies show that dehydrogenase, phosphoesterase, monoamine oxidase, and cholinesterase activities are unaffected. However, the paraformaldehyde-fluorescence method reveals that the tunica adventitia of migrainous arteries develops a striking increase in its binding capacity for *noradrenaline* after incubation of sections in this catecholamine.

It could be argued that this increased binding capacity is an adaptive response to repeated attacks of migraine involving the same segment of the temporal artery—such as had occurred in the patients from whom the biopsies were taken. This response could be considered as a local mechanism for storing *noradrenaline* to overcome the segmental dilatation of the migrainous temporal artery.

However, the increased adventitial capacity to bind *noradrenaline* in the migrainous artery is difficult to explain in terms of such a secondary response, for an increased level of *noradrenaline*

in the arterial wall, such as is produced by injection of monoamine oxidase inhibitors, leads to the appearance of demonstrable extraneural catecholamine in the tunica media rather than the tunica adventitia (Doležel, 1966). Moreover, an adaptive catecholamine-storing response would be expected to be accompanied by hyperplasia of adrenergic nerve fibres, but no such neural proliferation was observed in the migrainous artery.

The balance of evidence suggests that, in the migrainous artery, *noradrenaline* released from nerve terminals at the adventitio-medial junction (see Adams, 1967) is unavailable to act on its target-tissue—smooth-muscle in the tunica media—because it is bound by collagen in the tunica adventitia. Nevertheless, Gillespie (1966) reported that, while vascular smooth-muscle binds *noradrenaline*, normal elastin and collagen do not have this capacity.

Failure of *noradrenaline* to penetrate and act on the smooth-muscle of the tunica media would encourage dilatation of the arterial wall. Dilatation is characteristic of the *temporal* arteries in migraine, but the *intracranial* arteries are thought first to constrict and then dilate (Wolff, 1963). From the information available, the present very tentative hypothesis must, therefore, be restricted to events in the temporal arteries.

The anti-depressive agent desipramine (and related compounds) are known to impede *in vitro* *noradrenaline* uptake by adrenergic fibres around the rat aorta (see Results), by various nuclei in the rat brain and by adrenergic fibres in the rat vas deferens (Carlsson *et al.*, 1966; see Glowinski and Axelrod, 1964). However, preincubation with this drug does not prevent *in vitro* uptake of *noradrenaline* by the tunica adventitia of the migrainous human temporal artery.

It may not be entirely fortuitous that migrainous headache responds to ergotamine. Moreover, the prophylactic use of a suitable ergotamine preparation can be very effective in avoiding migraine; the amine methysergide is also useful in this respect. However, ergotamine in our short-term *in vitro* studies did not affect the binding of *noradrenaline* by the arterial adventitia, so the success of the drug must be attributed to some other local or general mechanism.

In conclusion, it is clear that further studies are required in less florid and less chronic forms of migraine to determine whether or not the adventitial catecholamine-binding in migrainous temporal arteries is a primary or secondary event in this condition.

SUMMARY

The temporal arteries were biopsied in six patients while they were suffering from an acute attack

of migraine and who complained of localized pain over a segment of the vessel. These biopsies reveal no significant structural change in the vessel wall. Histochemical methods do not disclose any significant change in dehydrogenase, phosphoesterase, monoamine oxidase, and cholinesterase activities in these arteries. However, the paraformaldehyde-fluorescence method shows that the tunica adventitia of migrainous arteries has a marked capacity to bind noradrenaline. This change may be a secondary response to repeated attacks of arterial dilatation in migraine. On the other hand, it may be a primary pathogenic event in migraine. The tentative hypothesis is presented that such adventitial catecholamine-binding may prevent noradrenaline from acting on the medial smooth-muscle fibres in the dilated migrainous temporal artery.

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ADDENDUM

It was pointed out to us during discussion at the Second Migraine Symposium (National Hospital for Nervous Diseases, London, 24 November 1967) that the migrainous arteries were obtained under local anaesthesia with procaine, whereas the control vessels were removed under general anaesthesia. In view of this comment we investigated the effect of incubating two necropsy specimens of

temporal arteries in 2% procaine: this procedure did not increase the amount of subsequent noradrenaline-fluorescence in the tunica adventitia of these vessels.

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