PAPERS

Beta-adrenoceptor and epoprostenol (prostacyclin) responsiveness of lymphocytes in migraine patients

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Summary

Beta-adrenoceptor blocking drugs are of prophylactic value in some patients with migraine. Beta-adrenoceptor responsiveness as measured by lymphocyte cAMP responsiveness to isoprenaline has, therefore, been compared in ten untreated classical migraine patients and ten matched non-migraine control subjects. Lymphocyte responsiveness to epoprostenol (prostacyclin, PGI₂) was also compared. No difference in response to either agent was found between the two groups. A generalized abnormality of beta-adrenoceptor function does not appear to be present in classical migraine, but a defect localized to specific vascular regions cannot be excluded.

KEY WORDS: isoprenaline, cyclic AMP.

Introduction

The relationship between cardiovascular, neurological and haematological factors in the pathogenesis of migraine is not understood nor, consequently, is the mode of action of prophylactic migraine treatments. As a preliminary study to examination of beta-adrenoceptor and PGI₂ receptor functional status during prophylactic treatment of classical migraine with beta-blockers, we have examined the general responsiveness of these vasodilating receptor systems during attack free periods. Beta-adrenoceptor and PGI₂ receptor responsiveness was examined on lymphocytes by measuring isoprenaline and PGI₂ stimulated cAMP elevation. Beta-adrenoceptor density was also measured.

Patients

Ten patients experiencing migraine attacks with a definite prodromal syndrome were admitted into the study. Patients with signs of cardiovascular, respiratory or other neurological disorders were excluded. All patients were untreated and had received no medication for at least 2 weeks before investigation. Ten healthy non-migrainous subjects, matched with the patients for age and sex formed the control group.

Methods

Cell preparation

Peripheral blood was heparinized (20 units/ml) and immediately diluted with an equal volume of phosphate buffered saline (PBS) supplemented with glucose (5.55×10^{-3} M). Diluted plasma was removed by centrifugation for 30 min at 150 g and 20°C. The remaining cells were rediluted with PBS to replace the volume of diluted plasma removed. Lymphocytes were separated by a modification of the method of Böyum (1968). Diluted blood (14 ml) was layered over 10 ml ficoll-paque (Pharmacia Ltd, U.K.) and centrifuged for 30 min at 400 g and 20°C. The lymphocyte buffy coat was removed and diluted with five volumes of PBS. The lymphocyte suspension was then centrifuged for 10 min at 150 g and 12°C. The remaining cell pellet was resuspended, rediluted with six volumes of PBS and centrifuged for 30 min at 400 g and 12°C. The cells were resuspended in PBS and cell counting was performed using Turk's stain. In all cases granulocyte contamination was less than 1% and the platelet:lymphocyte ratio was no greater than three.

Isoprenaline and PGI₂ incubation

Lymphocytes (2×10^6 /ml) were incubated with isobutylmethylxanthine (5×10^{-4} M) and isoprenaline (10^{-9} – 10^{-4} M) or PGI₂ (10^{-11} – 10^{-4} M) for 15 min at 37°C. Incubation was terminated by immersion in boiling water for 5 min, after which EDTA was added to a final concentration of 5×10^{-3} M. The cell debris was removed by centrifugation at 2×10^3 g for 30 min at 20°C and the supernatant removed for cAMP assay and stored frozen at –25°C. cAMP content was assayed using a modification of the method of Brown *et al.* (1971) in which 100 µl supernatant samples were incubated with 25 µl ³H cAMP (1.8×10^{-8} M; 40 Ci/mmol) and 200 µl bovine adrenal extract prepared as previously described (Brown *et al.*, 1971), for 2 hr at 1–4°C. The bound fraction was separated with addition of 500 µl charcoal suspension (20 mg activated charcoal/ml; 3 mg bovine serum albumin/ml) at 1–4°C and centrifuged at 2.5×10^3 g for 10 min at 1–4°C.

Receptor binding studies

Previously frozen lymphocytes were homogenized ice cold with an Ultra-Turrax homogenizer for 30 s. The membrane suspension was centrifuged for 30 min at 1.5×10^4 g and 1–2°C. The supernatant was discarded and replaced with incubation buffer (Tris 0.01 M, NaCl 0.15 M, bovine serum albumin (5×10^{-6} g/ml) at 1°C. Membranes of 2×10^5 lymphocytes were incubated with (–)¹²⁵I-iodocyanopindolol (ICYP) (Amersham, U.K.) at 10 – 200×10^{-12} M in 200 µl at 37°C for 1 hr. Nonspecific binding was determined by inclusion of (±)isoprenaline HCl (2×10^{-4} M) and ascorbic acid (10^{-3} M) in the incubation. Incubations were terminated by dilution with 10 ml of incubation buffer at 37°C and immediate filtration through Whatman GF/C filters. The filters were washed with 10 ml of incubation buffer at 37°C and counted in a gamma counter.

Results

No difference in responsiveness of the lymphocytes to either isoprenaline or PGI₂ was observed at any concentration. The beta-adrenoceptor binding studies demonstrated no difference between the two groups with respect to beta-adrenoceptor density (B_{max}) or affinity as reflected in the dissociation constant (K_D) derived from Scatchard analysis. Dose response curves to isoprenaline and PGI₂ are shown in Fig. 1 and 2 respectively, and results of beta-adrenoceptor binding studies are summarized in Table 1.

Discussion

Some beta-adrenoceptor blocking drugs, particularly those such as propranolol, atenolol and sotalol which lack partial agonist activity, have been shown

TABLE 1. β-adrenoceptor binding data

	B_{max} (binding sites/ cell ± s.e.m.)	K_D (pM ± s.e.m.)
Control subjects (n = 10)	1082 ± 95.1	71.85 ± 12.8
Migraine patients (n = 9)	1047 ± 83.1	108.1 ± 13.1

FIG. 1. Isoprenaline-induced cAMP accumulation in lymphocytes of migrainous and control subjects (●—●) control subjects (n = 10) (■—■) migraine patients (n = 10).

to reduce the incidence and severity of migraine attacks when used prophylactically in some patients (Weerasurya *et al.*, 1982). The mechanism underlying this therapeutic effect is not known, but it is reasonable to suppose that beta-adrenoceptors are involved in some way. If there is a generalized beta-adrenoceptor abnormality in migraine, then it might be expected to be present in the lymphocyte. A reduction in lymphocyte responsiveness has been demonstrated in patients with essential hypertension (Lima and Turner, 1982) and anxiety states (Lima, 1983), conditions in which beta-blocking drugs are of therapeutic value. The results of the present study have failed to demonstrate any difference between lymphocyte beta-adrenoceptor responsiveness and number when compared with matched control subjects, and it is unlikely, therefore, that a general defect of beta-adrenoceptor function exists in migraine. A local defect is not excluded, however. Essential tremor is readily amenable to treatment with beta-blocking drugs, and beta-adrenoceptors may be involved in its production, at least in part, but lymphocyte beta-adrenoceptor function is not abnormal in patients with essential tremor (Kilfeather *et al.*, 1984). It is possible, therefore, that while some

FIG. 2. Prostacyclin-induced cAMP accumulation in lymphocytes of migraineous and control subjects (●—●) control subjects ($n=10$); (■—■) migraine patients ($n=10$).

pathological conditions in which beta-blockers are of therapeutic value may be associated with generalized changes in beta-adrenoceptor function, others are not, but are due to changes localized to particular tissues. It may also be argued that as only a proportion of migraineous patients are helped by beta-blocking drugs, a disorder of beta-adrenoceptor function might only be found in them rather than in an unselected population such as were studied here. Further studies are indicated, therefore, in a larger population of patients in which response to beta-blocking drugs is determined under controlled conditions and pretreatment lymphocyte beta-adrenoceptor responses are related to response to treatment.

Following the observation by Lima and Turner (1982) that treatment of patients with essential hypertension with beta-receptor blocking drugs was accompanied by increasing lymphocyte responses not only to isoprenaline, but also to PGI₂, Weerasurya *et al.* (1982) suggested that treatment of migraine with beta-adrenoceptor blocking drugs might lead to increased sensitivity to PGI₂. This awaits investigation, but the present study failed to demonstrate any generalized change in responsiveness to PGI₂ in migraine patients compared with matched non-migrainous control subjects. Once again, however, the possibility of changes localized to the cerebral vasculature cannot be excluded, and it is also possible that a generalized defect might be detected in patients who are known to be responsive to beta-adrenoceptor blocking drugs. This will be the subject of future investigations.

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