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Increased platelet aggregation and release reaction in myotonic dystrophy

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SUMMARY

Platelet aggregation (PA) induced by (–)-epinephrine and adenosine diphosphate (ADP) was studied in 16 patients with myotonic dystrophy (MyD) and 14 healthy subjects. Plasma β-thromboglobulin level (β-TG), a useful marker of in vivo platelet release reaction, as well as in vitro 5-[^{14}C]hydroxytryptamine (5-HT) release, were also studied. The extent of PA induced by (–)-epinephrine at 1 and 3 min and by ADP at 3 min was significantly higher in the patients than in controls. Plasma β-TG and ADP- or epinephrine-induced platelet 5-HT release were also increased in MyD patients. These results suggest that an abnormality in release as well as in α_2-receptor functioning occurs in the platelets of MyD patients. The relation of this abnormality to changes in Ca^{2+} fluxes through the platelet membrane is discussed.

Key words: Myotonic dystrophy; Platelet aggregation; Release reaction; Adrenergic receptors

INTRODUCTION

Myotonic dystrophy (MyD) is an autosomal dominant disease characterized by sustained contraction of skeletal muscle fibres which persists after stimulation has

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ceased (Harper 1979). Most patients demonstrate dysfunction of other organs including myocardial conduction defects, gonadal atrophy, ocular cataracts, frontal baldness and glucose intolerance (Harper 1979; Roses et al. 1979). The multi-system involvement has suggested that the primary abnormality in MyD is a generalized membrane dysfunction (Atkinson et al. 1980). Increased epinephrine-induced platelet aggregation (with a normal response to ADP and collagen) has been reported in MyD patients (Bousser et al. 1975). We have found that in normal subjects (–)-epinephrine induced platelet aggregation (PA) is associated with a decrease in basal platelet cAMP level (Zahavi et al. 1985), presumably through binding to an α_2-adrenergic receptor and the inhibition of membrane adenylcyclase (Alexander et al. 1978; Mukherjee 1981). The increased platelet sensitivity to epinephrine in MyD, therefore, might indicate an abnormality of the α_2-adrenergic receptor. However, the increased platelet sensitivity in MyD was not confirmed by other investigators (Bolhuis et al. 1982).

The controversy over PA in MyD patients (Bousser et al. 1975; Bolhuis et al. 1982) urged us to extend the study of platelet function in these patients. The specific platelet protein β-thromboglobulin (β-TG) is found in the plasma after the occurrence of the release reaction, and it is a known marker of in vivo platelet activation and 5-[^{14}C]HT release (Shapira and Hutton 1985). In addition to measuring plasma β-TG levels we have also estimated the in vitro 5-[^{14}C]HT released from platelets by epinephrine and ADP. Concurrently, we have also measured PA using a wide concentration range of epinephrine and ADP. We report here an enhanced platelet function in MyD patients.

MATERIALS AND METHODS

Sixteen patients (12 males and 4 females, mean age 34.4 years) and 14 apparently healthy subjects (9 males and 5 females, mean age 34.3 years) were studied. The diagnosis of MyD was established by a positive family history, clinical evaluation and typical electromyographic response. Patients and normal subjects abstained from the use of platelet suppressive drugs for 2 weeks.

Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared from citrated (0.11 M) whole blood as previously described (Lanza et al. 1987; Cella et al. 1978). Concentration of platelets was standardized to 2×10^8 platelets per ml by diluting PRP with PPP. PRP was stirred at 1 100 rpm and at 37 °C for 1 min before PA inducers were added and reaction was performed for another 3 min during which transmission was recorded (Fig. 1). 5-[^{14}C]HT release from platelets was performed by a modification of the method of Zucker et al. (Born 1962; Kruglak et al. 1984; Dvilansky et al. 1976). PRP (2×10^8 platelets/ml) was incubated with 5-[^{14}C]HT for 20 min at 37 °C. Then 0.5 ml of the radioactive PRP was incubated in the Payton aggregometer at conditions already described for PA, after ADP or epinephrine was added. Samples were withdrawn at 2 and 4 min and diluted twice with ice-cold 50 mM EDTA. Centrifugation was performed for 1 min in an Eppendorf centrifuge (15 000 × g) and the supernatant counted. Plasma β-TG was measured by radioimmunoassay (Ludlam et al. 1975) using Amersham kits (Amersham Radiochemical Centre, U.K.).

Statistical analysis

Mann-Whitney U-test and Wilcoxon paired differences test were used in the analysis of the PA and release reaction data. To obtain a normal distribution of plasma β-TG values, a log-normal transformation was performed, and Student's *t*-test was used (Siegel 1956).

RESULTS

Plasma β-TG level

Plasma β-TG values were higher in the patients (mean 56 ng/ml, range 18–245) than in controls (mean 27 ng/ml, range 18–71.5) with an overlap of the values. Statistically the difference between the mean logarithmic values of β-TG of the 2 groups was significant ($P = 0.023$, Student's 2-tailed *t*-test).

Platelet aggregation (PA)

Fig. 1 shows the change in transmission with time after addition of 0.2 μM (A) or 1 μM (B) of (–)-epinephrine or following addition of 0.5 μM (C) or 1.5 μM (D) ADP in a patient's or a control subject's PRP. It can be seen that in the patient 2 waves (2 maxima) of PA are observed (up to 1 min and then at 3 min) while in the control only one maximum is found (at 1 min with epinephrine and at 0.5 min with ADP). Also in controls 2 waves of PA can be obtained if the concentration of PA inducers is raised.

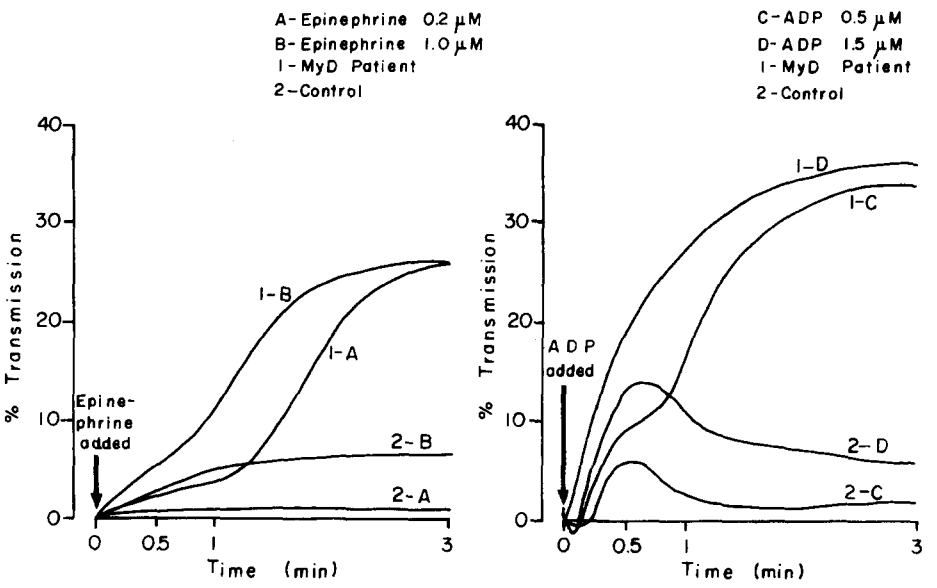

Fig. 1. Epinephrine and ADP-induced platelet aggregation in a patient with myotonic dystrophy (MyD) and in a normal control. Light transmission (%) was standardized as PPP was set to 45 or 95% transmission (2 channels) and PRP to 5 or 55%. For demonstration reasons, the patient with PA in the upper range was selected.

Some patients exhibit only one wave of PA, especially at lower PA inducer concentrations. High concentrations of PA inducers and longer times of PA (here 3 min) are likely to produce the "second wave of PA" which is thought to indicate release of granules, while lower concentrations and shorter times (0.5–1 min) will give the first maximum which represents PA per sec.

Epinephrine-induced PA was higher in the patients than in controls (Table 1). The difference was not significant for 0.2 μM, but was pronounced at 0.3–1 μM (particularly the extent at 3 min; Table 1). The extent of ADP-induced PA (0.5–1.5 μM) was also higher in the patients than in controls, reaching statistical significance at high ADP concentrations (Table 2).

Platelet release reaction

5-[^{14}C]HT release, an indicator of in vitro platelet dense bodies (β-granules) release induced by ADP or (–)-epinephrine was significantly higher in the patients than in controls at submaximal concentrations. At higher concentrations there was no significant difference between the 2 groups (Table 3).

DISCUSSION

This study provides evidence for elevated plasma β-TG in MyD patients, indicating enhanced in vivo platelet activation and release reaction. This may result from the presence of young platelets in the circulation. This observation may be related to the relative thrombocytopenia observed in MyD (Lanza et al. 1987). It should also be noted that young platelets contain more granular substance (α-granules and dense bodies) and are functionally more active than mature or old platelets. Such young platelets are more sensitive to PA inducers and 5-HT release is increased in them (Karpatkin 1972). Thus it is possible that a higher turnover rate of platelets occurs in MyD. Another possible explanation, however, is that the generalized membrane dysfunction, postulated by other investigators as the basic abnormality in MyD (Atkinson et al. 1980), may result in enhanced platelet sensitivity to PA inducers. As can be seen in Results, however, PA is primarily increased in response to epinephrine, which might suggest involvement of the α_2 adrenergic receptor in the platelet membrane. This observation supports previous reports (Bousser et al. 1975). Through its binding to the α_2-adrenergic receptor, (–)-epinephrine may inhibit membrane adenylcyclase and thereby induce PA and release reaction. The mechanism of this inhibition may be direct or indirect through changes in calcium influx (Owen et al. 1980). It has been reported that at low concentrations (–)-epinephrine can affect calcium influx into the platelets (Owen et al. 1980), thus triggering PA and the release reaction (Vermylen et al. 1983), although this view has been contested (Lanza et al. 1987).

The greater sensitivity of MyD platelets to low concentrations of epinephrine may be part of an enhanced platelet Ca^{2+} uptake mechanism in MyD patients. Indeed, in addition to α_2-receptor abnormality in MyD patients an alternative explanation of our

TABLE 1

PLATELET AGGREGATION (MEAN AND RANGE) IN RESPONSE TO VARIOUS CONCENTRATIONS OF (−)-EPINEPHRINE IN MyD PATIENTS AND CONTROLS

$E_{0.5, 1, 3}$ = extent of aggregation in arbitrary chart divisions 0.5, 1, and 3 min after the addition of (−)-epinephrine.

	0.2 μM			0.3 μM			1.0 μM		
	Controls	Patients	P^a	Controls	Patients	P^a	Controls	Patients	P^a
$E_{0.5}$	1.5 (1–3)	1.45 (0.5–3)	NS	1.3 (0–2.5)	2.0 (1–5)	<0.05	2.1 (1–3)	3.2 (1.5–7)	<0.045
E_1	2.0 (1–4)	2.3 (0.5–4)	NS	2.45 (1–5)	3.5 (1–6.5)	<0.037	4.8 (2–9)	6.1 (1.5–9)	NS
E_3	3.2 (1.5–5.5)	4.7 (1–27.5)	NS	3.4 (1.5–6.5)	4.7 (1.5–9.5)	<0.05	4.3 (2.5–9)	12.3 (2–23)	<0.01

[a] Mann–Whitney U-test (one-tailed) comparing 15 MyD patients to 12 controls; NS = not significant.

TABLE 2

PLATELET AGGREGATION (MEAN AND RANGE) IN MyD PATIENTS AND CONTROLS IN RESPONSE TO ADENOSINE DIPHOSPHATE (ADP) CONCENTRATIONS

$E_{0.5, 1, 3}$ = extent of aggregation in arbitrary chart divisions 0.5, 1, and 3 min after the addition of ADP.

	0.5 μM			1.0 μM			1.5 μM		
	Controls	Patients	P^a	Controls	Patients	P^a	Controls	Patients	P^a
$E_{0.5}$	4.8 (2–9)	5.9 (1–7.5)	NS	9.2 (4.5–15)	10.5 (6–14)	NS	11.1 (7–15)	11.2 (6–17)	NS
E_1	2.8 (1–8)	3.8 (0–5.5)	NS	8.1 (3.5–15)	10.8 (3–25)	NS	11.5 (5.5–23)	15.8 (7–24)	<0.017
E_3	2.5 (0.5–7)	4.1 (0–31)	NS	6.5 (1–22)	9.1 (0–33)	NS	8.4 (1–32)	19.7 (10–39)	<0.024

[a] Mann–Whitney U-test (one-tailed) comparing 15 MyD patients to 12 controls; NS = not significant.

TABLE 3

5[^{14}C]HYDROXYTRYPTAMINE RELEASE IN MyD PATIENTS AND CONTROLS

Legend as in Table 1. R_2, R_4 = percent release (mean and range) 2 and 4 min, respectively, after the addition of the aggregation inducer.

	(−)-Epinephrine, 1.5 μM			(−)-Epinephrine, 2.5 μM				Adenosine diphosphate, 1.5 μM			Adenosine diphosphate, 2 μM		
	Controls	Patients	*P*	Controls	Patients	*P*		Controls	Patients	*P*	Controls	Patients	*P*
R_2	5.9 (0–35.5)	6.5 (0–50)	<0.05	8.0 (0–55.5)	13.5 (0–63)	NS	R_2	3.9 (0–31)	6.5 (0–33)	<0.05	6.8 (0–40.5)	13.9 (0–81.1)	NS
R_4	11.35 (0–47)	17.6 (0–51.7)	<0.035	35.4 (2.1–92.6)	30.1 (0–62.5)	NS	R_4	5.4 (0–22)	16.5 (0–62.4)	<0.022	20.4 (0–51.1)	19.3 (0–55.5)	NS

data on PA is possible: the first maximum for ADP-induced PA is reached already at 0.5 min while epinephrine-induced PA is still increasing at 1 min (Tables 1 and 2; Fig. 1). Therefore, epinephrine-induced PA may be more sensitive for demonstrating, at our conditions, differences between MyD patients and controls. The difference between the induced PA in speed of attaining maximum PA may be inherent in the difference in their mechanism of induced PA, for instance, epinephrine induced PA and 5-HT release may require ADP release from β granules (Owen et al. 1980).

Our results with respect to PA differ from those reported by other investigators (Bolhuis et al. 1982; Lanza et al. 1987). Bolhuis et al. (1982) found no difference between MyD patients and controls. However, in their study EDTA was used for the isolation of platelets, and this agent might inhibit platelet activity (Vermylen et al. 1983) and thereby desensitize PA. Bousser et al. reported increased platelet sensitivity to (−)-epinephrine, but not to ADP, in MyD patients (Bousser et al. 1975). These investigators used only low ADP concentrations in their study, and in this range their results were similar to ours. Only by using higher ADP concentrations, have we observed that the extent of ADP-induced PA was also significantly increased (Table 2).

When this paper was in preparation other investigators confirmed that platelet function (plasma β-TG and A23187 calcium ionophore induced PA, but not ADP- or epinephrine-induced PA) was enhanced in MyD patients (Shapira and Hutton 1985). Lanza et al. (1987) reported normal aggregation in response and 5-HT release but gave no quantitative results. In fact, according to their data, epinephrine enhanced thrombin-induced 5-HT release more in MyD than in controls. The investigators concluded that the difference was not statistically significant. However, they have used Student's t-test which may be invalid under the conditions studied.

We have found that in vivo platelet release reaction and in vitro 5-HT release were enhanced in our patients. Since the extent of PA at three minutes (which is mostly an indicator of the release reaction, particularly at high inducer concentration) was also increased, it is conceivable that platelet release reaction is the primary platelet abnormality in MyD.

Our results thus provide evidence that platelet function is abnormal in MyD patients, but the underlying mechanism is still unknown. However, if the postulated abnormal calcium influx through the platelet membrane in MyD is confirmed, this could be corrected by the use of calcium channel blockers. We have found that nifedipine inhibits PA and even more the release reaction in normal subjects (Zahavi and Zahavi 1985). Other investigators obtained similar results with verapamil (Addonizio et al. 1982). It is pertinent to mention that there are marked similarities between the actomyosin of muscle and the thrombostenine of platelets (Lusher and Bettex-Guland 1972). Also, the process of phosphorylation of myosin-like membrane proteins during platelet release is similar to the myosin-actin contraction process (Haslam et al. 1978; Adelstein et al. 1978). In addition there are marked similarities between the storage of 5-HT in the β-granules of platelets (Fukami and Sulganicoff 1977) and of neurotransmitters in synaptic vesicles (Sneddon 1973) and calcium is intimately involved in the platelet release reaction and neurotransmitter release. The enhanced release which we found in platelets of MyD patients may be paralleled by abnormalities in calcium-mediated processes in other excitable tissues.

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