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THE ROLE OF MYOGENIC RELAXATION, ADENOSINE AND PROSTAGLANDINS IN HUMAN FOREARM REACTIVE HYPERAEMIA

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SUMMARY

1. Forearm blood flow was measured bilaterally in healthy young male and female volunteers, in the basal state and after upper-arm occlusion of arterial or venous blood flow for 1–20 min. The investigations were repeated after pre-treatment with drugs affecting vascular prostaglandins and/or adenosine.

2. Simultaneous arterial occlusion in one arm and venous occlusion in the contralateral arm for up to 20 min elicited a considerable reactive hyperaemia in the arm subjected to arterial occlusion, but completely failed to elevate the post-occlusive flow in the arm subjected to venous occlusion above the pre-occlusive level.

3. When the arterial occlusion was increased from 1 to 20 min there was a progressive increase in the subsequent reactive hyperaemia, up to 30 ml 100 ml tissue^{-1}. The time dependence following 1–3 min of arterial occlusion was based on a facilitation of the peak post-occlusive flow, while prolongation of the arterial occlusion from 3 to 20 min augmented the reactive hyperaemia mainly by increasing its duration.

4. Inhibition of prostaglandin synthesis with ibuprofen reduced the total reactive hyperaemia following 3–5 min of arterial occlusion by up to 70 %. This attenuation was due both to a reduction of peak post-occlusive flow and to a shortening of the duration of the post-occlusive hyperaemia.

5. The adenosine receptor antagonist theophylline reduced the reactive hyperaemia following 5 min of arterial occlusion by about 35 %. Combined treatment with ibuprofen and theophylline did not reduce the reactive hyperaemia more than either drug alone.

6. Infusion of dipyridamole, a drug which inhibits the elimination of adenosine, reinforced the reactive hyperaemia by about 45 %. This effect of dipyridamole was completely inhibited by administration of theophylline, and also by ibuprofen.

7. Plasma levels of adenosine, hypoxanthine and uric acid were maintained during the reactive hyperaemia, indicating increased production of purines during or immediately after the ischaemia.

8. It is concluded that the adequate stimulus for vascular relaxation in response

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to interruption of blood flow is omission of vessel wall distension. Local metabolic factors like endogenously formed prostaglandins and adenosine may act synergistically to this myogenic response but seem to be inactive alone. The lack of additive effects of ibuprofen and theophylline suggests a link between vascular relaxation induced by prostaglandins and by adenosine.

INTRODUCTION

Temporary arrest of the circulation through a limb is followed by a short-lasting augmentation of flow, usually referred to as reactive hyperaemia. Reactive hyperaemia is thought to be based on an interplay between physical and local metabolic factors (cf. Shepherd, 1963; Folkow & Neil, 1971; Sparks & Belloni, 1978). Accordingly, the basal myogenic tone is due to continuous stretching, elicited by the systemic blood pressure, of the vascular smooth muscle cells (Bayliss, 1902). The hyperaemia appearing even after a few seconds of arterial occlusion would, hence, be explained by vascular smooth muscle relaxation due to lack of wall stretch in the occluded and collapsed arterial tree.

The metabolic part of the reactive hyperaemia has not been completely classified. Inhibition of prostaglandin formation considerably diminishes reactive hyperaemia in animals (Herbaczynska-Cedro, Staszewska-Barczak & Janczewska, 1974; Messina, Weiner & Kaley, 1974, 1977), and in humans (Kilbom & Wennmalm, 1974, 1976; Nowak & Wennmalm, 1979; Carlsson & Wennmalm, 1983), suggesting that at least a significant part of the metabolic component is elicited by locally formed vasodilator prostaglandins. Another factor suggested in this connection is the ATP degradation product adenosine (Cerretelli, 1969; Dobson, Rubio & Berne, 1971; Tabaie, Scott & Hardy, 1977).

In order to further characterize the mechanism(s) behind reactive hyperaemia we tried to study separately the myogenic (physical) and the metabolic contributions to the total post-occlusive vascular relaxation. We assumed that such a distinction would be achieved via (a) separate and combined variations of the occlusion pressure and time, and (b) pharmacological interference with some endogenous factors, i.e. adenosine and prostaglandin, that may contribute to the metabolic component of reactive hyperaemia.

METHODS

Subjects

Altogether sixteen healthy normotensive male and female subjects (age 19–41 years), most of them hospital staff, participated in various series in the investigation. None of them were on medication for any kind of cardiovascular, endocrinological or other major disease, and none of them had taken any asprin-like drug during the week preceding either of the steps in the study. The subjects were not allowed to smoke or drink tea or coffee during the 4 h preceding each step in the investigation.

All subjects were carefully informed about the nature, purpose and possible risks involved in the study before giving their written consent. The protocol was approved by the local human investigation's committee.

Measurement of forearm blood flow

A 2-channel device, allowing parallel measurement of right and left forearm blood flow, was constructed at our department. The changes in forearm volume induced by proximal venous

occlusion were measured with an air-filled (pressure = 3 mmHg) latex rubber cuff (Nolato, Torekov, Sweden) placed around the thickest part of the forearm (Dohn, 1956; Graf & Westersten, 1959). The venous occlusion pressure (applied for measurement of arterial blood flow, cf. below) was 60–70 mmHg. Cuff pressure changes induced by venous occlusion were measured with a pressure transducer–amplifier–recorder system (EMT 33, EMT 31, Mingograph; Siemens-Elema, Stockholm, Sweden). Air inflation into the cuff was used for calibration. The blood flow to the hand was not occluded during the measurements since pilot experiments clearly showed that such occlusion did not change the recorded amplitude of either basal or post-occlusive forearm blood flow.

The basal forearm blood flow was estimated as the mean of four to six occlusions. The post-occlusive flow was measured every 15 s for 180 s after a proximal upper-arm occlusion. Arterial occlusion was induced by inflating a tourniquet around the upper arm to a pressure of 250 mmHg. Venous occlusion (applied for induction of ischaemia as an alternative to arterial occlusion, cf. above) was induced by inflating the tourniquet to a pressure of 100 mmHg. All recordings were performed in supine position. Room temperature (20 ± 1 °C), air humidity (70 ± 5 %) and air velocity were kept constant during all recordings.

Since the initial experiments demonstrated a non-significant sex and a significant side difference in the magnitudes of the reactive hyperaemia care was taken to include equal numbers of males and females in each series and to study equal numbers of right and left forearms among them. Comparisons between series were only performed if the same forearm of the same subject was included in both series.

Protocol

Effect of variations in occlusion pressure and time on reactive hyperaemia. The reactive hyperaemia following 1, 3, 5, 10 or 20 min of arterial or venous occlusion was randomly studied in the right or left forearm in each subject. The recordings were separated by an interval of at least 15 min at the end of which the basal flow recording was performed.

Effect of drugs. The effect of ibuprofen on reactive hyperaemia following 1–20 min of arterial occlusion was studied by repeating the protocol 1–7 days after the basal recordings, 1 h after peroral administration of ibuprofen.

The reactive hyperaemia following 1–10 min of arterial occlusion was also reinvestigated immediately after an I.V. infusion of theophylline. In addition an investigation was performed, 1–7 days later, to study the combined effect of ibuprofen and theophylline (given as above) on the post-occlusive flow following 5 min of arterial occlusion.

The effects of dipyridamole were studied twice on three different occasions. Initially, basal flow and reactive hyperaemia were studied before and 30 min after an I.V. infusion of dipyridamole. A catheter for blood sampling was inserted in the brachial artery of the arm not to be studied. A venous sampling catheter was inserted 5–8 cm in a retrograde direction into a medial cubital vein in the arm to be studied. Arterial and venous blood samples were drawn in the basal state and immediately following release of the arterial occlusion, before and during treatment with dipyridamole.

On the second occasion, the subjects were initially given an I.V. infusion of theophylline. Basal and post-occlusive blood flows were measured 20–30 min after the infusion. Then dipyridamole was administered as described above. After the end of the dipyridamole infusion an additional dose of theophylline was given. Basal and post-occlusive blood flows were determined 30 min after terminating these infusions.

On the third occasion, the subjects had taken ibuprofen 1 h before the flow measurements. After basal and post-occlusive blood flow recordings, dipyridamole was given as above. Thereafter, the basal and post-occlusive flow recordings were repeated.

The efficacy of ibuprofen as an inhibitor of the prostaglandin synthesizing enzyme cyclooxygenase was checked using arachidonate-induced aggregation of platelets in platelet-rich plasma sampled before and during treatment with the drug (i.e. on the first and third occasion).

Plasma analyses

Samples for determination of adenosine, hypoxanthine and uric acid were collected as described by Sollevi, Östergren, Fagrell & Hjemdahl (1984). Blood was sampled in pre-cooled syringes containing an equal volume of saline containing dipyridamole (20 μM), indomethacin (60 μM), heparin (4 mg l^{-1}), erythro-9-(2-hydroxy-3-nonyl)-adenine (10 μM) and an internal standard (dimethylguanosine). After separation of plasma, the proteins were precipitated with 0·4 M-perchloric

acid and sedimented at 1000 *g* for 5 min. The supernatant was neutralized with 2·5 M-ammonium acetate (pH 9·5) and stored at −70 °C until analysis. The samples were purified with affinity chromatography (phenylboronate) and analysed using high-performance liquid chromatography with adsorbance detection (Fredholm & Sollevi, 1981). Dipyridamole levels in plasma were analysed according to Pedersen (1979), using methoxydipyridamole as internal standard.

Drugs

Ibuprofen (Brufen, Boots, Nottingham, U.K.) was given as a single oral dose of 3 × 400 mg, 1 h before the flow recording. Theophylline (Teofyllamin, ACO, Solna, Sweden) was given as an I.V. infusion at a dose of 6 mg kg^{-1} during 20 min. The additional dose given between the flow recordings was 2 mg kg^{-1} and it was given as above. Dipyridamole (Persantin, Boehringer-Ingelheim, Ingelheim, F.R.G.) was given as an I.V. infusion at a dose of 0·4 mg kg^{-1} during 30 min before the second flow recording.

Calculations

At each individual investigation a blood-flow–time curve was constructed. Total post-occlusive flow was estimated as the area under this flow–time curve during minute 0–3 after release of the arterial or venous occlusion. Total reactive hyperaemia was calculated as the total post-occlusive flow, minus the basal flow during this period. As a measure of the basal flow during the hyperaemia period, the preceding flow was used. The peak post-occlusive flow represents the highest flow figure obtained during the 3 min following the release of the arterial occlusion. The duration of the reactive hyperaemia is given as the half-time, i.e. the period after which reactive hyperaemia, i.e. peak post-occlusive flow minus basal flow, was reduced by at least 50 %.

All flow figures are given in ml 100 ml $tissue^{-1}$ min^{-1}. Values for total reactive hyperaemia are given as ml 100 ml $tissue^{-1}$ (referring to the entire 3 min period following release of the arterial or venous occlusion). For calculation of statistical differences Student's *t* test for paired means (two-tailed) has been used, when applicable. All values in the text, Tables and Figures are given as mean ± S.E. of the mean.

RESULTS

The basal forearm blood flow in the different series varied between 2·6 ± 0·2 and 6·3 ± 1·2 ml 100 ml $tissue^{-1}$ min^{-1}. No sex or side differences in the basal forearm blood flow were observed.

Sex and side differences in total reactive hyperaemia

The possible sex and side differences in reactive hyperaemia were analysed in experiments utilizing 5 min of arterial occlusion. In males such occlusion resulted in a total reactive hyperaemia that averaged 6·5 ± 1·4 ml 100 ml $tissue^{-1}$. The corresponding figure for females was 8·5 ± 2·1 ml 100 ml $tissue^{-1}$ The difference is not significant.

When equal numbers of male and female left forearm total reactive hyperaemia data were pooled the average figure was 6·8 ± 1·4 ml 100 ml $tissue^{-1}$. The corresponding figure for the right forearm was 8·5 ± 1·4 ml 100 ml $tissue^{-1}$. The difference is significant ($P = 0·043$).

Effect of venous vs. arterial occlusion

When the occlusion tourniquet was inflated to a pressure of 100 mmHg to induce venous instead of arterial occlusion, no post-occlusive change in blood flow was detectable after either 5, 10 or 20 min of venous occlusion. Simultaneously recorded reactive hyperaemia in the contralateral arm subjected to arterial occlusion of similar duration, was all the expected magnitude (Fig. 1).

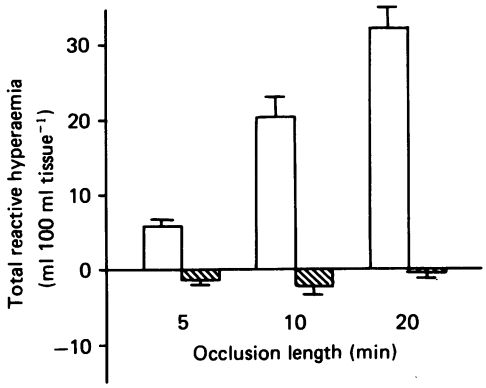

Fig. 1. Total forearm reactive hyperaemia (post-occlusive–basal forearm blood flow) after release of arterial and contralateral venous upper-arm occlusion maintained for 5, 10 or 20 min. Equal numbers of right and left upper-arm arterial occlusions were included in all series. Open columns, arterial occlusion; hatched columns, venous occlusion.

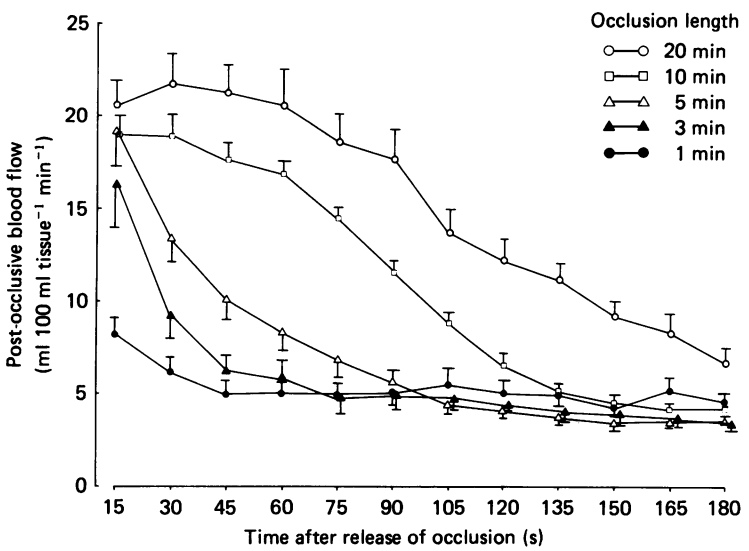

Fig. 2. Post-occlusive forearm blood flow in healthy volunteers during the 3 min immediately following upper-arm arterial occlusion maintained for 1, 3, 5, 10 or 20 min.

Effect of occlusion time

Arterial occlusion maintained for 1 min resulted in a small total reactive hyperaemia, about 0·5 ml 100 ml tissue^{-1} (Figs. 2 and 3). Prolongation of the arterial occlusion resulted in an almost linear increase in the total reactive hyperaemia, to about 30 ml 100 ml tissue^{-1} after 20 min of occlusion (Fig. 3). The increment to the total reactive hyperaemia after 3 as against 1 min of arterial occlusion mainly came from an augmentation of the peak post-occlusive flow, as is evident from Figs. 2 and 4. In contrast, when occlusion was further prolonged from 3 to 20 min, the hyperaemic increment largely reflected an increased half-time of the post-occlusive

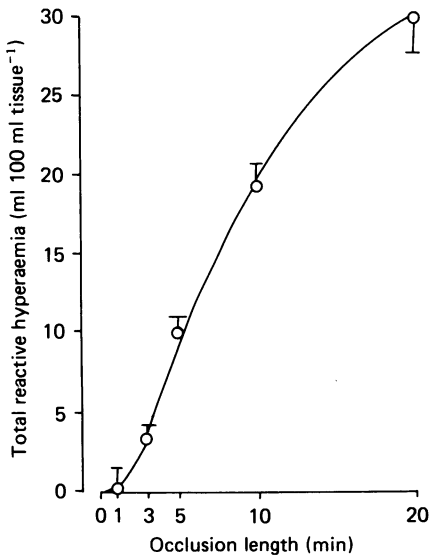

Fig. 3. Relation between the length of the arterial occlusion and the resulting forearm reactive hyperaemia (post-occlusive–basal forearm blood flow).

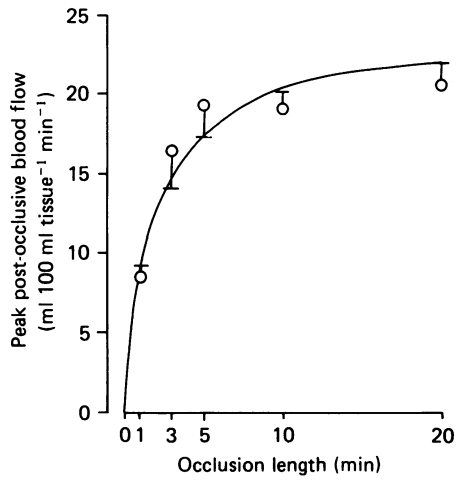

Fig. 4. Relation between the length of the arterial occlusion and the resulting peak post-occlusive forearm blood flow.

hyperaemia, as seen from Figs. 2 and 5, the peak post-occlusive flow being almost the same after 3 as after 20 min of occlusion (Figs. 2 and 4).

Effect of drugs

The basal forearm blood flow in the ibuprofen series was $6{\cdot}3 \pm 1{\cdot}2$ ml 100 ml tissue^{-1} min^{-1}; it was unaffected by ibuprofen treatment ($6{\cdot}5 \pm 1{\cdot}2$ ml^{-1} 100 ml tissue^{-1} min^{-1}).

After administration of ibuprofen the total reactive hyperaemias in response to 3

and 5 min periods of arterial occlusion were significantly reduced ($P<0.01$). A numerical but not statistically significant decrease in total reactive hyperaemia was also obtained after 10 and 20 min of arterial occlusion (Table 1). The reduction in total reactive hyperaemia after 3 and 5 min of arterial occlusion came from an

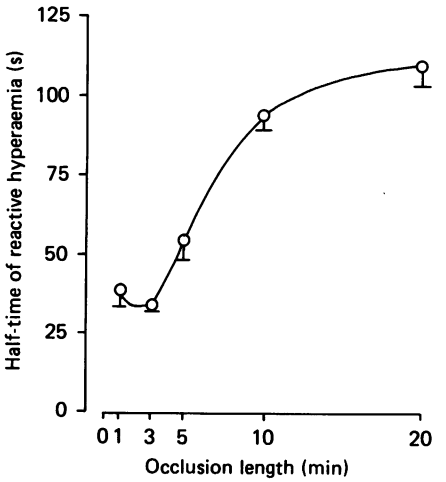

Fig. 5. Relation between the length of the arterial occlusion and the half-time of the resulting forearm reactive hyperaemia. The half-time is expressed as the length of the period after release of upper-arm arterial occlusion, at which reactive hyperaemia, i.e. peak post-occlusive–basal forearm blood flow, was reduced by at least 50 %.

attenuation of the peak post-occlusive flow, as well as a shortening of the post-occlusive hyperaemia (Table 1, Fig. 6).

The basal forearm blood flow in the theophylline series was 4.8 ± 0.5 ml 100 ml^{-1} min^{-1} before and 4.3 ± 0.5 ml 100 ml tissue^{-1} min^{-1} after theophylline. These levels do not differ significantly. Arterial occlusion for 1, 5 and 10 min elicited post-occlusive increases in blood flow of mainly the same magnitude as in the ibuprofen series (cf. above). When the recordings were repeated after theophylline infusion the reactive hyperaemia following 5 min of occluson was inhibited by about 35 % ($P<0.005$, Fig. 7). The reactive hyperaemias following 1 and 10 min of arterial occlusion were also numerically lower in the presence of theophylline, in comparison to control, but these differences did not reach significance (Fig. 7). Combined treatment with ibuprofen and theophylline also reduced the reactive hyperaemia following 5 min of arterial occlusion by about 35 %. The combined treatment did not reduce the reactive hyperaemia more than either drug alone (Fig. 7).

The basal forearm blood flow in the dipyridamole series was 4.4 ± 0.6 ml 100 ml tissue^{-1} min^{-1}. Treatment with dipyridamole caused a plasma drug level of 0·64 μM (range 0·40–0·85 μM) during the occlusion period. Dipyridamole did not change the basal forearm flow, either alone or after previous administration of theophylline, but after pre-treatment with ibuprofen dipyridamole did induce a borderline ($P<0.05$) attenuation of the basal forearm blood flow (Table 2). In the absence of any drug total reactive hyperaemia in response to 10 min of arterial occlusion was about

TABLE 1. Basal (pre-occlusive) forearm blood flow, peak post-occlusive flow, duration of reactive hyperaemia and total reactive hyperaemia in healthy volunteers subjected to upper-arm arterial occlusion maintained for 1, 3, 5, 10 and 20 min. All recordings were repeated 1 h after oral administration of ibuprofen, a prostaglandin synthesis inhibitor, at a dose of 1200 mg

Duration of arterial occlusion (min)	Basal flow (ml 100 ml^{-1} min^{-1})		Peak post-occlusive flow (ml 100 ml^{-1} min^{-1})		Half-time of reactive hyperaemia(s)		Total reactive hyperaemia (ml 100 ml^{-1})	
	Control	After ibuprofen	Control	After ibuprofen	Control	After ibuprofen	Control	After ibuprofen
1	8·2±0·9	7·2±1·0	6·2±0·8	5·5±0·8	39±6	39±6	0·2±1·2	−0·2±0·9 (n.s.)
3	4·4±0·5	4·9±0·9	16·3±2·3	11·5±1·7**	49±5	34±2**	3·4±0·8	1·0±1·3**
5	9·4±0·3	10·7±1·4	24·9±2·1	20·1±1·7**	65±7	54±6*	13·4±1·1	3·8±2·0**
10	5·0±1·2	4·4±0·5	22·9±1·9	19·9±1·8	81±6	79±5	20·7±2·4	16·2±1·0 (n.s.)
20	4·3±0·7	5·2±1·0	23·6±1·5	17·9±1·5	122±10	107±6	32·2±2·5	24·5±3·3 (n.s.)

* and ** denote that the value is significantly lower ($P<0·05$, and 0·01, respectively, Wilcoxon's ranked sign test) than the corresponding value before drug; n.s. indicates that the value is not significantly different from the corresponding value before drug.

TABLE 2 Effect of dipyridamole (0·4 mg kg^{-1} I.V.), alone or after pre-treatment with theophylline (6 mg kg^{-1} I.V) or ibuprofen (1200 mg orally), on basal (pre-occlusive) forearm blood flow, as well as on the peak post-occlusive flow and total reactive hyperaemia following release of an upper-arm arterial occlusion maintained for 10 min

Flow variable	No drug		After theophylline		After ibuprofen	
	Control	After dipyridamole	Control	After dipyridamole	Control	After dipyridamole
Basal blood flow (ml 100 ml^{-1} min^{-1})	5·3±0·7	4·1±0·8	4·6±0·9	4·3±0·8	3·4±0·6	2·3±0·3
Peak post-occlusive flow (ml 100 ml^{-1} min^{-1})	21·4±1·9	24·6±1·7	20·7±1·9	21·5±1·6	19·7±3·5	16·9±2·7
Total reactive hyperaemia (ml 100 ml^{-1})	19·1±2·5	27·2±2·5**	16·1±2·5	17·5±1·9	17·8±3·7	18·0±3·7

** indicates that the value is significantly ($P<0·01$) higher than the corresponding value before dipyridamole.

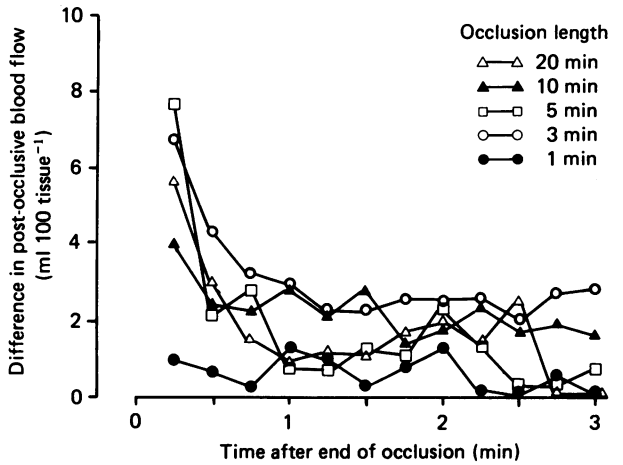

Fig. 6. Effect of ibuprofen (1200 mg orally), a prostaglandin synthesis inhibitor, on forearm blood flow during the 3 min following release of an upper-arm arterial occlusion maintained for 1, 3, 5, 10 or 20 min. The flow values represent the difference between post-occlusive flow values recorded before and after pre-treatment with the drug.

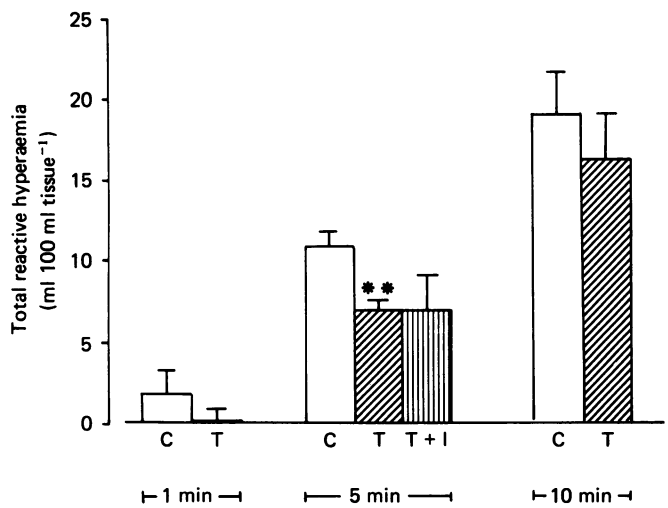

Fig. 7. Total forearm reactive hyperaemia after upper-arm arterial occlusion maintained for 1, 5 or 10 min. Open columns (C) represent the reactive hyperaemia before drug treatment, diagonally hatched columns (T) the values obtained after pre-treatment with theophylline (an adenosine receptor antagonist, 6 mg kg^{-1} I.V.), and the vertically hatched column (T+I) the total reactive hyperaemia after combined pre-treatment with theophylline (as above) and ibuprofen (a prostaglandin synthesis inhibitor, 1200 mg orally).

19 ml 100 ml $tissue^{-1}$. When dipyridamole was infused prior to the arterial occlusion the reactive hyperaemia increased compared to before drug by about 45% (Table 2, Fig. 8). The augmentation of the reactive hyperaemia came from a prolongation of its duration, the half-time being prolonged from 84 ± 6 to 98 ± 6 s ($P < 0.05$). Theophylline in itself exerted an insignificant effect on the total reactive hyperaemia

but completely abolished the potentiation induced by dipyridamole (Table 2). Pre-treatment with ibuprofen also failed to affect the magnitude of the post-occlusive hyperaemia, while completely abolishing the increase in total reactive hyperaemia induced by previous infusion of dipyridamole (Table 2).

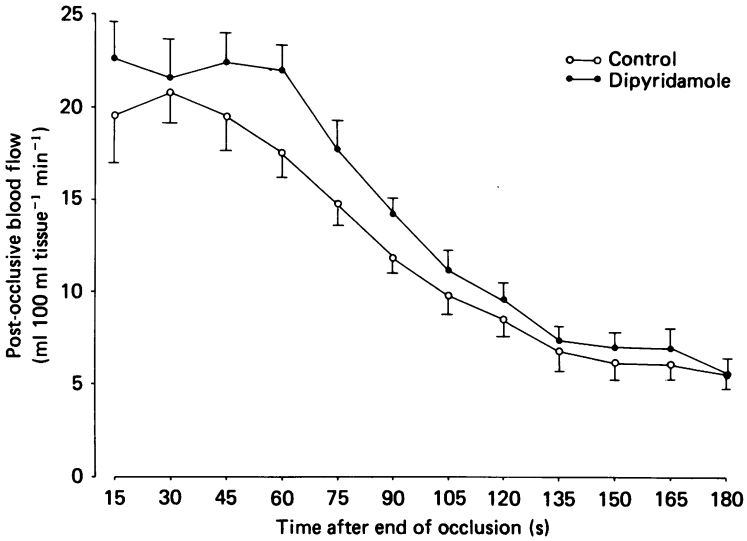

Fig. 8. Post-occlusive forearm blood flow during the 3 min following release of an upper-arm arterial occlusion maintained for 10 min. Open circles represent controls, performed in the absence of drug, and filled circles the recordings performed after pre-treatment of the subjects with dipyridamole (an antagonist of adenosine elimination, 0·4 mg kg^{-1} I.V.).

Arachidonate (50–100 μM) regularly induced platelet aggregation in platelet-rich plasma prepared from subjects not given ibuprofen. After administration of the drug, tenfold higher concentrations of arachidonate invariably failed to induce platelet aggregation, indicating that platelet cyclo-oxygenase was completely inhibited.

Plasma levels of adenosine and its metabolites

The basal arterial plasma level of adenosine was about 0·15 μM. There was no significant net release of adenosine into the venous effluent (Table 3). The basal arterial levels of hypoxantine and uric acid were about 3 and 250 μM respectively. In analogy with adenosine no significant net release of these adenosine metabolites was present (Table 3). After infusion of dipyridamole no definite change occurred in the plasma levels of adenosine or its metabolites.

Following 10 min of arterial occlusion the arterial plasma levels of adenosine and its metabolites were maintained at the basal levels, as expected. Venous levels were not lowered either, in spite of the marked increase in forearm blood flow, indicating that the turnover of adenosine and its metabolites quantitatively was augmented in parallel to the increase in forearm blood flow during the post-occlusive hyperaemia.

TABLE 3. Arterial and deep cubital venous plasma levels of adenosine, hypoxanthine, and uric acid in healthy subjects in the basal state and immediately following release of an upper-arm arterial occlusion maintained for 10 min, before and after I.V. infusion of dipyridamole (0·4 mg kg^{-1}). Values given in μM as mean ± S.E.

	Basal		After ischaemia	
	Arterial	Venous	Arterial	Venous
		Before dipyridamole		
Adenosine	0·14 ± 0·03	0·10 ± 0·02	0·16 ± 0·04	0·18 ± 0·06
Hypoxanthine	2·87 ± 0·31	3·12 ± 0·48	3·28 ± 0·54	3·84 ± 0·60
Uric acid	249 ± 26	257 ± 32	279 ± 33	290 ± 42
		After dipyridamole		
Adenosine	0·10 ± 0·01	0·14 ± 0·03	0·12 ± 0·02	0·16 ± 0·02
Hypoxanthine	3·32 ± 0·43	3·12 ± 0·42	3·37 ± 0·39	3·40 ± 0·49
Uric acid	272 ± 36	271 ± 36	265 ± 27	267 ± 34

DISCUSSION

We here report that venous occlusion maintained in one arm for up to 20 min is unable to elicit reactive hyperaemia, although simultaneous contralateral arterial occlusion elicits a post-occlusive increase in flow that is proportional to the length of the preceding occlusion. Venous occlusion can be assumed to elicit the same metabolic effects in the ischaemic tissue as arterial occlusion, since blood flow is completely inhibited in both cases. In contrast, venous occlusion should not elicit any myogenic relaxation because the normal stretching of the arterial vascular tree is maintained during the occlusion. Venous occlusion should consequently unmask the metabolic component of the reactive hyperaemia, a component that can be assumed to be more important the longer the occlusion time.

Although the current data from the arterial occlusion series seem to support such a view, those from the venous occlusion experiments oppose it. The pressure applied in the upper arm tourniquet in the latter experiments (100 mmHg) was most likely high enough to cause not only complete venous, but also partial arterial occlusion in the forearm. During systole the forearm arterial tree was probably opened by the pulse wave. This intermittent opening of the arterial tree was apparently sufficient to counteract completely reactive hyperaemia, both with respect to its myogenic and its metabolic components. It has been reported that in the anaesthetized cat the venous outflow from the hind leg is increased after release of arterial but not after venous occlusion (Hilton, 1953). The present results not only extend that observation to awake man, but also demonstrate that even long periods of venous occlusion are unable to elicit reactive hyperaemia.

The present data therefore question the conventional subdivision of reactive hyperaemia into a myogenic and a metabolic component. It rather seems that reactive hyperaemia basically is a myogenic phenomenon. The metabolic component, being a secondary phenomenon insasmuch as it cannot develop without the myogenic component, can probably prolong the myogenic relaxation or possibly delay the restoration of tension-induced vascular tone. It is, however, unable to dilate the vessels in the presence of maintained constant or intermittent vascular distension.

The increase in peak post-occlusive flow was very small when the occlusion was

prolonged from 3 to 20 min, despite parallel increases in the total reactive hyperaemia from less than 3 to more than 30 ml 100 ml tissue^{-1}. It was also clear from the present data that the increase in total reactive hyperaemia between 1 and 3 min of occlusion came entirely from an augmented peak post-occlusive flow; the half-time of the reactive hyperaemia did not differ between these two occlusion times. This accords with earlier data showing correlation between the occlusion length and the duration of the post-occlusive hyperaemia (Pattersen & Whelan, 1955). It has been claimed that reactive hyperaemia after brief arterial occlusion is mainly a myogenic phenomenon and that vasodilator metabolites hardly accumulate during occlusions lasting 1–3 min. If this is correct the current data indicate that the myogenic response in awake man is not fully developed until after about 3 min of arterial occlusion.

Right-arm reactive hyperaemia was larger in the present series than left-arm reactive hyperaemia; similar results have not been reported earlier. A possible explanation may be that the muscles in the right forearm more often are recruited for isometric work, which leads to ischaemia, and thereby have developed a more pronounced response to it.

Ibuprofen, a prostaglandin synthesis inhibitor, attenuated the hyperaemia responses to 3 and 5 min, but not 10 and 20 min of arterial occlusion. Arachidonate-induced aggregation of platelets was also inhibited by ibuprofen, indicating that the drug blocked platelet, and hence probably also vascular, cyclo-oxygenase. We suggest that the impairment of reactive hyperaemia was due to inhibition of the vascular formation of prostaglandin. Similar data have been reported earlier (Kilbom & Wennmalm, 1974, 1976; Nowak & Wennmalm, 1979; Carlsson & Wennmalm, 1983). The present data add that attenuation of reactive hyperaemia by prostaglandin synthesis inhibition is not independent of the duration of the arterial occlusion.

The basis for the decrease in importance of local prostaglandins after longer occlusion periods is not obvious. The release of prostaglandin precursor, i.e. arachidonic acid, may be time-limited or burst-like; if so the prostaglandin(s) initially formed during the occlusion would to some extent be metabolized, if sufficient time elapsed. Alternatively, oxygen pressure in the tissue may drop during prolonged arterial occlusion to a level at which the oxygenation of arachidonic acid is substrate (i.e. oxygen) limited.

Ibuprofen, after 3 and 5 min of ischaemia, inhibited both the peak post-occlusive flow and the half-time of the hyperaemic response, in comparison to control. This suggests that both the basal tone in the vascular smooth muscle (determining the flow in the absence of distensive pressure on vessel wall), i.e. peak post-occlusive flow, and the sensitivity of the vascular tone to the stretching force of the arterial pressure (determining the half-time of the reactive hyperaemia) are decreased following ischaemia by locally formed prostaglandins. Taken together, these two effects of ibuprofen indicate a drug-induced shift to the left in the vessel-distension–tone relationship and consequently that locally formed vasodilator prostaglandins shift this relation to the right.

Theophylline also decreased the total reactive hyperaemia following 5 min of arterial occlusion. The dose of theophylline administered is known to result in a plasma concentration sufficient to inhibit adenosine receptor activation (cf. Rall, 1980), also in man (Sollevi, Östergren, Fagrell & Hjemdahl, 1984). Thus, it seems that

endogenous adenosine also contributes to reactive hyperaemia. The role of locally formed adenosine in the development of reactive hyperaemia or ischaemic vasodilatation is controversial. Dobson *et al.* (1971) suggested that endogenous adenosine contributes to the metabolic regulation of skeletal muscle blood flow in a manner similar to that proposed for cardiac muscle (Rubio & Berne, 1969). In contrast, Hester, Guyton & Barber (1982), infusing adenosine to very high levels, were unable to change both reactive and exercise hyperaemia by such infusion, and concluded that such vasodilatation is caused either entirely or almost entirely by other factors than adenosine. The present clinical data seem to be the first to demonstrate a definite inhibitory effect of adenosine receptor blockade on reactive hyperaemia. The observation that both ibuprofen and theophylline displayed less effect following longer occlusion periods indicates that other vasodilator mechanisms gain in importance following longer periods of arterial occlusion.

Dipyridamole counteracts cellular adenosine uptake and thereby elevates its concentration in various tissues including human blood (Degenring, Curnish, Rubio & Berne, 1976; Sollevi & Fredholm, 1981; Sollevi *et al.* 1984). In the present experiments the drug's effect on reactive hyperaemia following 10 min of arterial occlusion was studied. This length of occlusion was chosen since theophylline and ibuprofen, which were to be used together with dipyridamole, lacked significant effects of their own on the reactive hyperaemia following such occlusion length. Dipyridamole failed to elevate the basal forearm blood flow but augmented the vascular response to arterial occlusion. This drug effect was completely abolished by theophylline, strongly suggesting that it was due to activation of adenosine-specific receptors in the vascular wall. Hence, the vascular response to 10 min of arterial occlusion can be potentiated by activation of adenosine-specific vascular receptors.

The potentiation of the reactive hyperaemia was blocked not only by theophylline but also by previous administration of ibuprofen. This is in accordance with the independent inhibitor actions of these two drugs on the reactive hyperaemia (without previous infusion of dipyridamole) elicited by 5 min of arterial occlusion, as discussed above. The similarity between these effects suggests a common basal mechanism, i.e. that adenosine acts as a vasodilator by a mechanism that in some way is linked to the local formation of some vasodilator prostaglandin. In this connection it is of interest that the formation of prostacyclin, a potent vasodilator prostaglandin of endothelial origin, in isolated rabbit hearts is considerably augmented by concentrations of adenosine that can be achieved under hypoxic or ischaemic conditions (Ciabattoni & Wennmalm, 1985). Further studies are, however, required to definitely establish a role for locally formed vasodilator prostaglandin in the vascular relaxation elicited by adenosine.

To summarize, the present study demonstrates that reactive hyperaemia in the human forearm basically is a myogenic phenomenon, elicited only in response to sustained and complete lack of vascular wall distension. Furthermore, the so-called metabolic response seems to reinforce the myogenic response but is unable to relax the vascular smooth muscle when wall tension is maintained or impaired only phasically.

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