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Different Influence of Endothelium in the Mechanical Responses of Human and Cat Isolated Cerebral Arteries to Several Agents

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Abstract—The present work was undertaken to elucidate the role of the vascular endothelium in the changes of isometric tension elicited by different compounds in isolated cylinders of human and cat cerebral arteries and cat pulmonary arteries. Endothelium removal by rubbing significantly reduced the relaxing response to acetylcholine (ACh) of isolated segments of all the arteries. The same treatment did not modify the contraction elicited by 5-hydroxytryptamine (5-HT) in the human and cat cerebral segments but increased the contractile effect of the amine in cat pulmonary arteries. The mechanical responses to vasopressin, ATP and adenosine in isolated segments of cat cerebral arteries were unaffected after removing the endothelial layer. L-Arginine, but not D-arginine (10^{-5} M), enhanced significantly the relaxation induced by increasing doses of ACh in unrubbed cat cerebral arteries whereas it did not modify the response to ACh in rubbed ones. However, L-arginine had no effect on the dose-response curve to 5-HT in both kinds of preparation and did not change the tone in precontracted unrubbed cat cerebral segments. These results suggest that the endothelium of the cerebrovascular bed plays a minor role in regulating the mechanical response induced by several vasoactive agents, although it retains its ability to produce an endothelium-derived relaxing factor.

There is increasing evidence that interactions between the endothelial cells and the underlying vascular smooth muscle play a role in controlling vasomotor tone. Furchgott & Zawadzki (1980) reported that acetylcholine (ACh) caused relaxation of precontracted strips of rabbit aorta only when endothelial cells were present. They suggested that the vascular endothelium released a labile substance, endothelium-derived relaxing factor (EDRF), which mediated the vascular response to several agents. It has also been suggested that endothelium-dependent agents may release different EDRFs depending on the vascular bed and animal species (Förstermann et al 1985; Rubanyi & Vanhoutte 1987). One of these has been identified as nitric oxide, NO (Palmer et al 1987). The precursor of NO seems to be L-arginine, since this amino acid increases NO synthesis in cultured endothelial cells from bovine and pig aorta (Palmer et al 1988; Schmidt et al 1988b). The formation of NO can be blocked by L-canavanine, an inhibitor of various L-arginine-utilizing enzymes, and by N^G-monomethyl L-arginine (L-NMMA), a structural analogue of L-arginine (Schmidt et al 1988b; Rees et al 1989). Furthermore, L-canavanine inhibits the endothelium-dependent relaxation elicited by ATP in rat thoracic rings whereas L-NMMA is able to abolish the vasodilation brought about by ACh in isolated segments of rabbit aorta (Schmidt et al 1988a; Rees et al 1989).

Many studies have confirmed the essential role of the endothelium in responses induced by various substances in a variety of peripheral preparations. However, attempts to show a functional role of these endothelial cells in isolated brain vessels have yielded conflicting results (Katusic et al 1984; Conde et al 1987; Hardebo et al 1987; Nakagomi et al 1988). While ACh seems to need the presence of the endothelium to exert its relaxing effect in all species studied, in the case of 5-hydroxytryptamine (5-HT) the role of endothelium is more dubious. Thus, 5-HT has been reported either to depend on the integrity of the endothelial cells for inducing vasoconstriction in rabbit and dog (Nakagomi et al 1988) or to be endothelium-independent for eliciting its contractile response in cat (Young et al 1986).

In previous work, we have found that the endothelium of cat cerebral arteries does not play a role in the mechanical response to platelets, this effect being mediated by 5-HT liberated as a result of their activation (Conde et al 1987). Therefore, it seemed important to determine whether vasoconstrictor or vasodilator responses induced by agents released during platelet aggregation might be influenced by the cerebrovascular endothelium.

To achieve this, we studied the effect of endothelium removal on the mechanical response of cat isolated cerebral arteries to ACh, 5-HT, vasopressin, ATP and adenosine. For comparative purposes, we analysed the influence of removing the endothelial cells on the effect induced by ACh and 5-HT in human isolated cerebral arteries and cat pulmonary arteries. To complete this, we also studied whether L-arginine was able to increase the production of EDRF in cat isolated cerebral arteries.

Materials and Methods

Thirty mongrel cats of either sex, 2–3 kg, were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.) and exsanguinated. The brain was carefully removed and both middle cerebral arteries (0·4–0·5 mm in outer diameter) dissected. Afterwards, the chest cavity was opened and pulmonary arteries (outer diameter approximately 1·0–1·5 mm) were

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dissected free of adjoining connective tissue and lung parenchyma.

Human cerebral vessels from 5 adults of either sex aged between 60 and 75 years were used. Autopsies were performed by the Pathology Service of La Paz Hospital (Madrid) 3 to 10 h after death. The cause of death was neither from cerebrovascular disease nor from tumours affecting the central nervous system. Branches of both middle cerebral arteries (0·8–1·0 mm in outer diameter) were dissected, quickly immersed in cooled physiological solution and transported to the laboratory. The arteries were freed from surrounding tissues under a dissecting microscope, and those with atheromatous changes were discarded. Thereafter, they were either used immediately or stored in Krebs-Henseleit solution at 4°C for not more than 24 h for use in subsequent experiments.

Isometric tension recording

Each artery was cut into paired cylindrical segments, 2–4 mm in length, with the aid of a dissecting microscope. The arteries were placed for isometric tension recording (Nielsen & Owman 1971) in 4–6 mL organ baths containing Krebs-Henseleit solution of the following composition (mM): NaCl, 115; KCl, 4·6; KH_2PO_4, 1·2; $MgSO_4.7H_2O$, 1·2; $CaCl_2$, 2·5; $NaHCO_3$, 25; glucose, 11·1 and EDTA, 0·03. The baths were kept at 37°C by means of a water jacket and bubbled continuously with a 95% O_2-5% CO_2 gas mixture to maintain the pH at 7·4.

Two stainless steel pins, 75 μm in diameter for cat cerebral segments and 150 μm for the other arterial tissues, were introduced carefully through the arterial lumen under an operative microscope to avoid damage to the artery. One pin was fixed to the organ bath wall while the other was connected to a strain gauge for isometric tension recording. The latter pin was parallel to the former and movable, allowing the application of resting tension at right angles to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3, a Statham Micro-Scale Accessory UL5 and a Beckman Type RS Recorder.

A resting tension was applied to the tissue and readjusted every 15 min during a 60–90 min equilibration period. The resting tension applied was 0·3 g for the cat cerebral arteries and 1·0 g for both the human cerebral arteries and cat pulmonary arteries.

Endothelial cells

Endothelium removal was achieved by gentle rubbing of the intimal surface with a stainless steel rod of appropriate diameter inserted through the lumen. The presence of endothelial cells was checked in each preparation by the relaxing response to ACh (10^{-7}–10^{-5} M) added on the plateau of the contraction induced by 4×10^{-8} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The failure of the vessel segments to relax upon the addition of ACh confirmed that the endothelium had been successfully removed; endothelium was considered to be present when the ACh-induced relaxation was greater than 60% (see Results). The bath medium was then renewed until the steady baseline tone was re-established.

Histological verification of the presence or loss of the endothelium was performed by cutting the artery longitudinally, and treating it with 0·4% silver nitrate, followed by a 10 min exposure to UV light. Afterwards, they were washed and fixed (Caplan et al 1971). On light microscopy, arteries with endothelium revealed a mosaic pattern of silver lines, which were considered to represent the endothelial cell borders. No mosaic pattern was seen in arteries whose intimal layers had been rubbed.

Dose-response curves

The dose-response curves were performed in a cumulative manner in the arterial segments with 5-HT (10^{-9}–10^{-5} M), vasopressin (10^{-11}–3×10^{-7} M), ACh (10^{-7}–10^{-5} M), adenosine 5′-triphosphate (10^{-7}–3×10^{-5} M), adenosine (10^{-8}–3×10^{-5} M) and L-arginine (10^{-7}–10^{-4} M). Relaxation was studied after the arterial segments had been contracted with $PGF_{2\alpha}$ (4×10^{-8} M).

The corresponding dose-response curves were obtained simultaneously in unrubbed and rubbed vascular segments.

In some cat cerebral segments L-arginine or D-arginine (10^{-5} M) was added to the bath medium after the dose-response curve to ACh had been obtained and 10 min later this was repeated without removing the amino acid from the medium. To control any change in sensitivity of the vascular segments to ACh, the same procedure was followed simultaneously in another group of arterial preparations but omitting the amino acid.

To study the effect of L-arginine (10^{-5} M) on the dose-response curve to 5-HT, the amino acid was added to the bath medium 10 min before the 5-HT experiment was performed and remained in contact with the tissue until its completion.

Statistical analysis

The data are expressed as means ± s.e.m., in mg of changes in tension. Statistical comparisons between vascular responses were made using Student's *t*-test. The geometric mean of ED50 with its 95% confidence interval was obtained as described by Fleming et al (1972). The Mann-Whitney U-test was used to compare the values of ED50. P values < 0.05 were considered to indicate significant differences.

Drugs

The following agents, all from Sigma, were used: acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate, arginine-vasopressin acetate, adenosine 5′-triphosphate, adenosine, L-arginine, D-arginine and prostaglandin $F_{2\alpha}$.

Concentrations of drugs are expressed as final molar concentration (M) in the bath medium.

Results

Responses to ACh

Unrubbed arterial segments previously contracted with $PGF_{2\alpha}$ from pulmonary and middle cerebral arteries of cat and from human cerebral arteries were able to relax when challenged with ACh (Figs 1, 2 left). Since there might be a partial removal of the endothelium by the pins for recording the changes in tension, only those segments showing a relaxation greater than 60% of the previous contraction were considered as possessing their endothelial cells undamaged

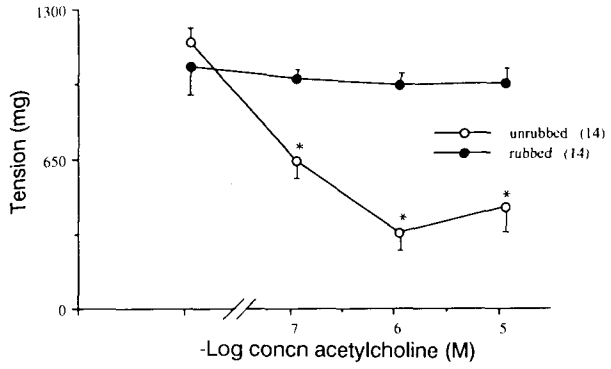

FIG. 1. Effect of rubbing on the relaxation elicited by ACh in cat isolated middle cerebral arteries. Each point represents the mean ± s.e.m. Figures in parentheses indicate the number of segments used. Asterisks denote statistical significance ($P < 0.001$).

and were used as control. The maximal relaxing response elicited by ACh related to the previous tone induced by $PGF_{2\alpha}$ was in each case: (i) 82 ± 8% (number of segments n = 10) over a previous tone of 1092 ± 100 mg for the human cerebral arteries, (ii) 75 ± 6% (n = 14) over a previous tone of 1134 ± 100 mg for the cat middle cerebral arteries, (iii) 97 ± 9% (n = 7) over a previous tone of 1293 ± 200 mg for the cat pulmonary arteries.

Cylindrical segments which had been gently rubbed with a stainless-steel rod showed a maximal relaxing response to ACh that reached about a 10% of the contraction previously obtained with the addition of $PGF_{2\alpha}$ (Figs 1, 2 left). At light microscopy, these segments were devoid of the typical mosaic pattern shown by the endothelial cells after staining with silver nitrate and under exposure to UV light (results not shown).

Responses to 5-HT

Isolated segments of cat cerebral and pulmonary arteries not submitted to rubbing showed a contractile response when exposed to increasing concentrations of 5-HT. The same result was obtained with human cerebral arteries. These responses to 5-HT were affected by rubbing only in the case of cat pulmonary arteries.

Fig. 2 shows a typical recording of a pair of segments from the same human cerebral artery. While endothelium removal abolishes the relaxation induced by ACh, it leaves the contractions elicited by 5-HT unaffected. The average dose-response curves to 5-HT obtained under these experimental conditions with isolated segments of human and cat cerebral arteries are shown in Fig. 3 (upper and middle plot, respectively). Neither the maximal response developed nor the ED50 were different in the presence or absence of endothelium. The value of the geometric mean of the ED50 with its 95% confidence interval was 1·5 (0·9–2·4) $\times 10^{-8}$ M (n = 10) for the human cerebral segments with endothelium and 2·2 (1·5–3·1) $\times 10^{-8}$ M (n = 16) for the cat isolated cerebral arteries with their endothelium intact.

In cat pulmonary arteries, low concentrations of 5-HT (10^{-8}–10^{-6} M) induced less contraction in unrubbed arteries than in rubbed ones ($P < 0.05$); at higher concentrations the contractile responses were similar for both groups of arteries (Fig. 3, lower plot). The ED50 for arteries with endothelium was 2·9 (0·9–9·8) $\times 10^{-7}$ M (n = 7) and that for preparations without endothelial cells 0·8 (0·3–1·6) $\times 10^{-7}$ M (n = 5); this difference was statistically significant ($P < 0.05$).

Responses to vasopressin

In cat cerebral arteries, vasopressin caused concentration-dependent contractions in all the segments tested. The ED50

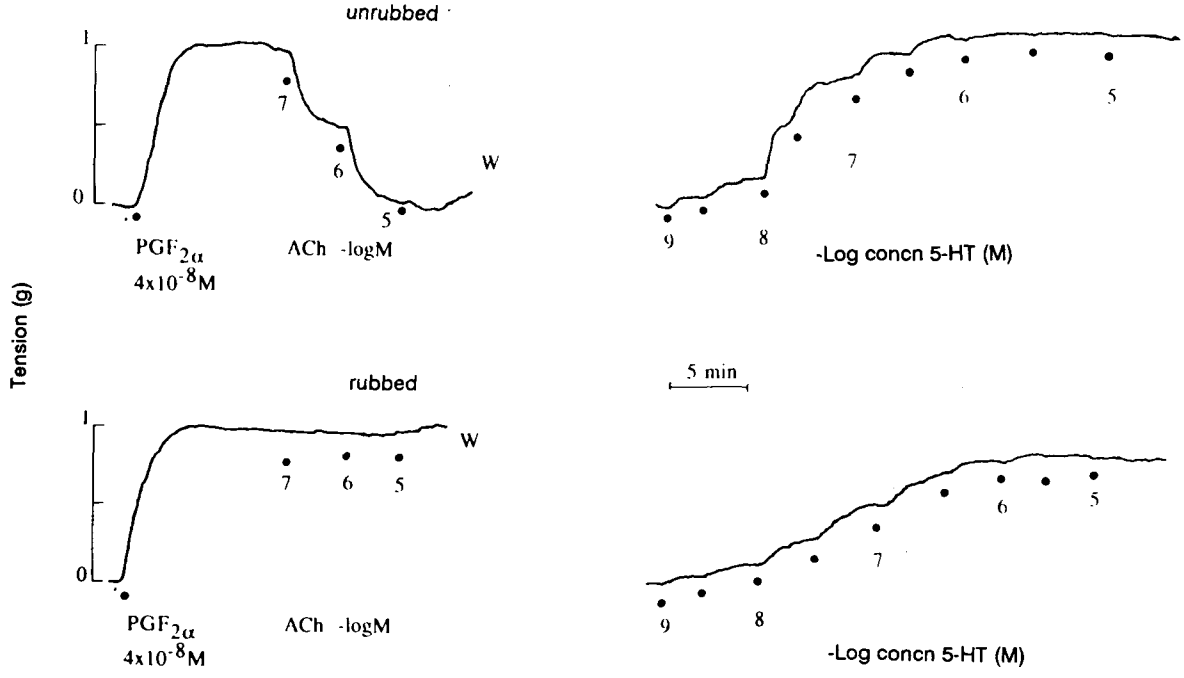

FIG. 2. Original tension recordings of two segments from the same human cerebral artery: one of them unrubbed (upper tracings) and the other one rubbed (lower tracings). After contracting them with $PGF_{2\alpha}$ (4×10^{-8} M), only the segment with endothelium was able to relax against ACh. The bath medium was then washed out (W), and cumulative dose-response curves to 5-HT performed.

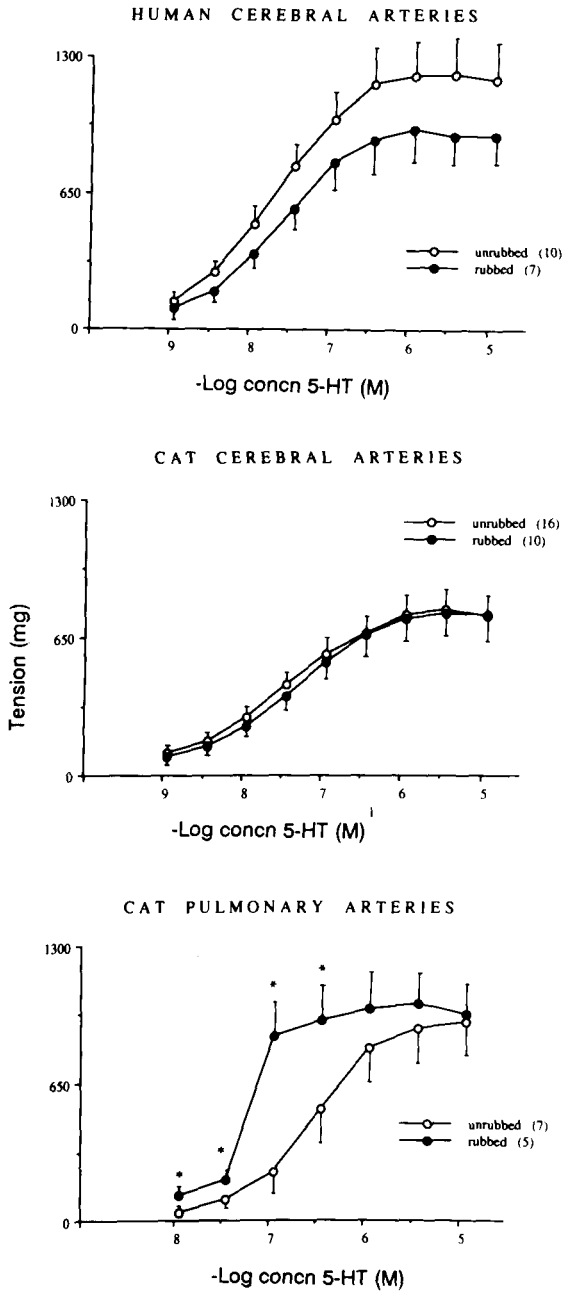

FIG. 3. Effect of endothelium removal on the contractile response induced by 5-HT in isolated segments of human cerebral arteries, cat cerebral arteries and cat pulmonary arteries. Each point represents the mean ± s.e.m. Figures in parentheses are the numbers of segments used. Asterisks denote statistical significance ($P < 0.05$).

value, 4·6 (1·2–17) $\times 10^{-10}$ M (n = 11), and the contractile effect of the peptide were not significantly changed by removal of the endothelium (Fig. 4, upper plot).

Responses to adenosine 5'-triphosphate

Precontracted cat cerebral arteries relaxed in a concentration-dependent way when exposed to ATP. Removal of the endothelium did not significantly affect the responsiveness and the ED50 value, 2·4 (1·7–3·2) $\times 10^{-6}$ M (n = 10), of these vessels to ATP (Fig. 4, middle plot).

Responses to adenosine

Adenosine caused concentration-dependent relaxation of cat cerebral arteries previously contracted. The absence of endothelial cells did not significantly modify the responsiveness and the ED50 value, 0·1 (0·2–8·2) $\times 10^{-7}$ M (n = 16), of the arteries to adenosine (Fig. 4, lower plot).

Effect of L-arginine

In unrubbed and rubbed isolated cat cerebral segment precontracted with $PGF_{2\alpha}$, L-arginine (10^{-7}–10^{-4} M) did not modify the corresponding tone.

The dilator response to ACh (10^{-7}–10^{-5} M) of unrubbed cat cerebral arteries was significantly increased ($P < 0.05$) in the presence of 10^{-5} M L-arginine (n = 17). When the arterial segments were submitted to rubbing no difference was found in the effect of ACh either in the absence or in the presence of L-arginine (Fig. 5, upper plot). On the other hand, the second exposure to ACh of the unrubbed vascular segments did not change its sensitivity to ACh.

The contractile dose-response curve to 5-HT of cat cerebral arteries with or without endothelium was not modified in the presence of 10^{-5} M L-arginine (Fig. 5, lower plot).

Effect of D-arginine

The addition of D-arginine (10^{-5} M) to the bath elicited a sustained contraction of cat unrubbed cerebral segments, reaching a tension of 78 ± 16 mg (n = 18). No statistical difference was found with the value obtained in rubbed arterial segments.

In the presence of D-arginine (10^{-5} M) there was a significant decrease in the dose-response curve to ACh performed in cat unrubbed cerebral segments ($P < 0.05$), whereas no difference was observed when unrubbed arterial segments were used (Fig. 5, middle plot).

The contractile tone previous to the addition of ACh in unrubbed segments was significantly higher in the presence of D-arginine than in its absence ($P < 0.05$).

Discussion

The present study was undertaken to gain more knowledge about the role of the cerebrovascular endothelium in the mechanical responses to substances which might be released during platelet aggregation. The available information on this matter is scarce and controversial. Thus, the endothelium seems to inhibit in-vitro the mechanical responses to: i) 5-HT in rabbit and dog (Nakagomi et al 1988), ii) ATP in rabbit, cat and man (Hardebo et al 1987) and iii) vasopressin in dog (Katusic et al 1984). On the other hand, endothelium-independent responses to 5-HT and adenosine have been shown in cat, rabbit and man (Young et al 1986; Hardebo et al 1987). The reason for such heterogeneity remains unclear.

The results reported here confirm the well-known influence of the endothelium on relaxation induced by ACh in isolated segments of blood vessels (Furchgott & Zawadzki 1980). This endothelium dependence seems to be widespread since it appears both in cerebral and pulmonary arteries as well as in cat and human cerebral blood vessels. Gentle rubbing of the arterial segments brought about a significant decrease in the relaxation induced by ACh. Such procedure

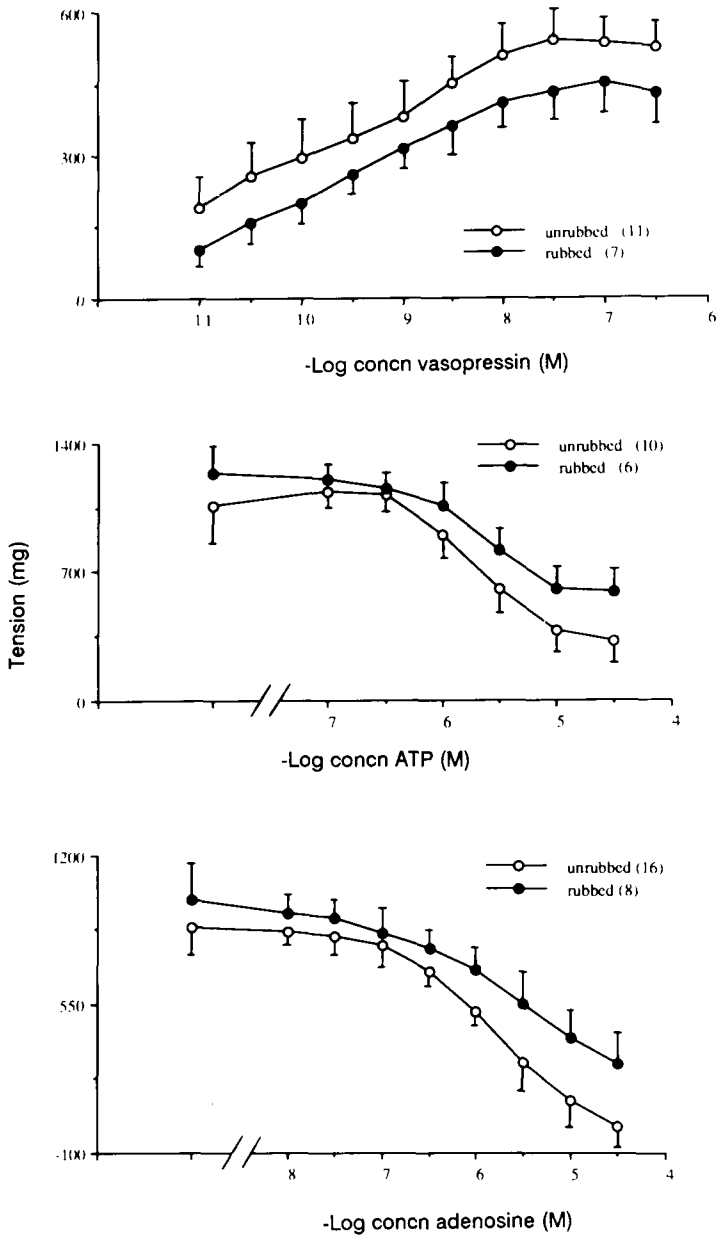

FIG. 4. Effect of rubbing the isolated segments of cat middle cerebral artery on the dose-response curves to vasopressin, ATP and adenosine. Each point represents the mean ± s.e.m. Figures in parentheses are the number of segments used.

removes the endothelial cell layer of the blood vessels, as confirmed from the histological evidence, without apparently affecting the mechanical properties of the muscle wall for there were no differences in the contractile response to 150 mM KCl between unrubbed and rubbed segments (results not shown).

The unrubbed cerebral segments of human origin showed a marked relaxing response to ACh not only 3–10 h after the death of the donor but even on the next day after storing them cooled. This suggests that the function of their endothelial cells is relatively long-lasting. This feature of the human cerebral arteries was shown in all the segments tested, partly disagreeing with Kanamaru et al (1989) who reported a more variable response of the human cerebral blood vessels. Another difference with the results of Kanamaru et al is the greater relaxation developed by the human arterial segments used here when exposed to ACh. Those authors could obtain a maximal relaxation of about 55% of the previous tone while in our experiments a vasodilation around 83% of the previous tone was reached. The discrepancies might be explained by methodological considerations such as the different anatomical origin of the arterial segments. In any case, our data indicate that autopsy specimens are suitable for studying endothelium-dependent responses in human isolated cerebral blood vessels.

On the contrary, no influence of the endothelial cells could be found in the mechanical responses of cat cerebral arteries to adenosine, vasopressin and ATP. The results obtained with adenosine agree with those published by Hardebo et al (1987) whereas the findings regarding vasopressin and ATP

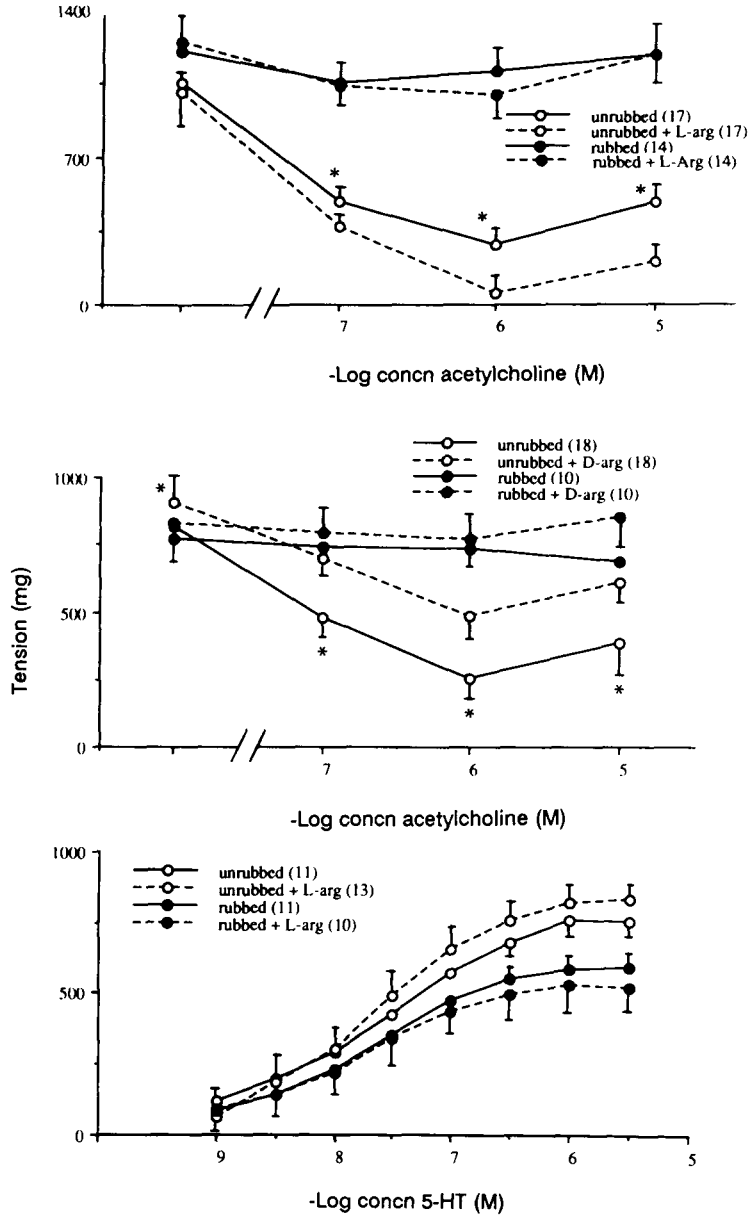

FIG. 5. Effect of 10^{-5} M L-arginine on the dose-response curves to ACh and 5-HT. The curves were performed in unrubbed and rubbed segments of cat middle cerebral artery. Each point represents the mean ± s.e.m. Figures in parentheses are the number of segments used. Asterisks denote statistical significance ($P < 0.05$).

differ from the evidence reported by other authors (Katusic et al 1984; Young et al 1986). In the case of vasopressin, the discrepancy between the results can be explained on the basis of the different animal species used. The relaxing response to ATP of cat isolated middle cerebral artery was found by Hardebo et al (1987) to be endothelium-dependent; however, the reasons for the differences in our results cannot be drawn from their published data.

Our data also indicate that the influence of endothelium on the contractile response to 5-HT differs depending on the vascular bed tested. While endothelium removal does not affect the contraction elicited by the amine in human and cat isolated cerebral arteries, it enhances the effect of 5-HT in cat pulmonary arteries. In addition to this, the role of endothelium on modulating the contractile response to 5-HT in the cerebral blood vessels seems to depend also on the animal species used, since an endothelium-dependence has been reported for rabbit and dog (Nakagomi et al 1988). This confirms the complexity of the endothelial cells' responsiveness (Vanhoutte & Miller 1985; Furchgott 1990).

It is possible that nitric oxide is the EDRF involved in the response to ACh. L-Arginine, which has been described as a precursor of nitric oxide (Palmer et al 1987; Schmidt et al 1988a), is able to potentiate the relaxation elicited by ACh in the isolated segments of cat middle cerebral artery. The effect of L-arginine disappears after rubbing the arterial segments and is a specific effect of this amino acid, since its D-isomer does not increase the response to ACh. However, L-arginine by itself cannot stimulate the production of EDRF. No change was observed when it was added at different doses to

precontracted segments or when its effect was studied on a response independent of endothelium such as that of 5-HT. The ability of L-arginine to increase the EDRF production could only be shown in the presence of an agent, ACh, that has its relaxing effect through the production and release of EDRF. This would indicate that L-arginine can act only when the production of EDRF has been already stimulated by ACh or by facilitating the action of ACh. Although the relaxation elicited by ACh in the presence of D-arginine is lower than in its absence, an inhibition of the production of EDRF by the amino acid seems very unlikely. It might be better explained on the basis that the contractile tone previous to the addition of ACh appeared enhanced in the presence of D-arginine, which possesses some contractile effect independent of endothelium.

In conclusion, our data show that the endothelium of the cerebral blood vessels behaves differently from that of peripheral arteries although it retains some ability to produce EDRF. The physiological meaning of such a discrepancy remains open.

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