Pharmacological Comparison of Isolated Human Cerebral and Digital Arteries

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SUMMARY Some basic pharmacological differences between isolated human cerebral and digital arteries obtained at postmortem have been studied. Spiral strip responses were measured isometrically and cumulative drug dose-response curves obtained. Contractile responses were sought to norepinephrine, 5-hydroxytryptamine, angiotensin, histamine, KCl, BaCl₂ and prostaglandin F₂α. Relaxation responses (either by the agonists on their own, or in the presence of a resting tonus induced by KCl or 5-hydroxytryptamine) were sought to isoproterenol, histamine, acetylcholine and dopamine.

There were no differences between the cerebral or digital arteries in the non-specific responses to KCl or BaCl₂. Nor were there any differences in the responses to prostaglandin F₂α, or in the receptor mediated responses to 5-hydroxytryptamine or angiotensin. This was in marked contrast to the responses produced by norepinephrine where the maximum response was much larger in digital arteries than in cerebral arteries, although there were no differences in the sensitivity of the 2 vessels to norepinephrine (as measured by the ED₆₀ values). Relaxation responses were difficult to elicit and very variable with only occasional preparations showing relaxation to any of the agonists.

These results suggest that the only major pharmacological difference between human digital and cerebral vessels is the number, rather than the sensitivity, of the noradrenergic α-receptors.

BLOOD FLOW to the brain and periphery often responds differently to various stimuli because of the differing physiological roles of these tissues. Yet little is known about the mechanisms of the response differences of cerebral and peripheral arteries to many factors, especially in man.

One of the possible explanations for differences in the responses of cerebral and peripheral arteries to various stimuli, would be altered post-junctional responses, either at the receptor level, or at the level of the smooth muscle contraction. This concept has been investigated in animals using isolated preparations. However, the considerable differences in pharmacological responses obtained between the various species make extrapolation to the human situation hazardous.

We have recently investigated the use of post-mortem human blood vessels to study the effects of vasoactive agents and have characterized in detail the responses of human digital arteries to different pharmacological stimuli. It has also been suggested that similarly obtained cerebral blood vessels may be suitable for use as a pharmacological preparation.

In order to gain more information about differences in regulation of blood flow between the cerebral and peripheral arterial beds in man, we have, therefore, compared and contrasted basic pharmacological properties of isolated human cerebral and digital arteries.

Materials and Methods

Collection of Arteries

Cerebral (usually the basilar) and common palmar digital arteries were removed at autopsy, between 3 and 60 (usually less than 30) h after death. A record was kept on each patient, including past medical history and treatment, cause of death and time after death before the arteries were removed.

Preparation of Arteries and Equipment

Immediately a cerebral or digital artery was obtained, a spiral strip was prepared as described by Jauernig and Moulds (1978) for digital arteries. The arteries were suspended in 30 ml tissue baths at 37° C, containing bath solution with the following composition (mmol.L⁻¹):

- Na⁺, 137.4; K⁺, 5.4; Ca²⁺, 2.5; Mg²⁺, 1.2; Cl⁻, 131.5; SO₄²⁻, 1.2; H₂PO₄⁻, 1.2; HCO₃⁻, 15.0; glucose, 11.5.

For experiments using barium chloride SO₄²⁻ was omitted from the bath fluid to avoid barium sulphate precipitation. Oxygen containing 5% carbon dioxide was gassed through the solution which then had a pH of about 7.4. Isometric tension was measured with either a Hewlett Packard FTA 100 or Grass FTO3C transducer and Hewlett Packard 7758A or 7702B recorder. Initial resting tension was adjusted to about 2 g and the arteries were left to relax for about 1½ h. Before the experiment proceeded, a final resting tension of approximately 1 g was obtained. Before each experiment, the response of the artery to 80 mmol.L⁻¹ potassium chloride (KCl) was determined and this contracture was used as the standard reference for that particular arterial segment, all agonist responses being expressed as a percentage of the KCl contracture in the respective artery.

Drugs

Drugs used were norepinephrine (L-arterenol bitartrate, Sigma), 5-hydroxytryptamine creatinine sulphate (Sigma), angiotensin amide (Hypertensin, Ciba), histamine dihydrochloride (Sigma), dopamine.
hydrochloride (Intropin, Anar-Stone Laboratories),
isoproterenol hydrochloride (Isuprel, Winthrop),
sodium nitroprusside (David Bull Laboratories),
sodium nitrite (Analar, British Drug Houses),
acetylcholine perchlorate (British Drug Houses),
prostaglandin \( \text{F}_2 \alpha \) (Upjohn Laboratories),
cocaine hydrochloride (May and Baker),
phenoxybenzamine hydrochloride, (Dibenylene, Smith, Kline & French),
barium chloride (British Drug Houses).

All drugs were prepared on the day of use.
Norepinephrine, isoproterenol, dopamine and
acetylcholine were diluted in ascorbic acid, \( 10^{-4} \) mol.\( l^{-1} \); other drugs were diluted in distilled \( H_2O \). All
agonist responses (both contractile and relaxant) were
studied by application of the agonist in cumulative
concentrations to the tissue bath.

Statistics

Data were statistically analyzed by a two-tailed
Student’s \( t \)-test. \( P \) values less than or equal to 0.05
were designated as being statistically significant.

Calculation of Mean \( \text{ED}_{50} \) Values

The median effective concentration (\( \text{ED}_{50} \), or
concentration of the agonist required to produce 50% of
the maximum response) of each agonist was
calculated from the concentration-effect curve for
each individual experiment. The \( \text{ED}_{50} \) concentration
(in mol.\( l^{-1} \)) was then converted to a negative
logarithm, calling this value the \( \rho \text{ED}_{50} \). From these
values the mean \( \pm \) the standard error of the mean
(SEM) was calculated, which thus served as a measure
of relative sensitivity between agonists.

Study of Relaxation Responses

A number of methods were used to investigate
relaxation responses to isoproterenol, acetylcholine,
histamine and dopamine. Firstly, agonists were
studied on their own. Secondly, because vascular
smooth muscle may be nearly completely relaxed in
vitro, responses were also studied after inducing a
resting tension in the artery. This was done by using
either 30 mmol.\( l^{-1} \) KCl or a special bath solution con-
taining 25.4 mmol.\( l^{-1} \) KCl (instead of 5.4 mmol.\( l^{-1} \)),
or with \( 10^{-4} \) mol.\( l^{-1} \) 5-hydroxytryptamine (5-HT), in
cases waiting for a stable contracture plateau
before testing the agonist. The relaxation was
expressed as a % of the standard KCl contracture.

Digital arteries were investigated first and because
of possible agonist cross-reaction with alpha-
adrenergic receptors, these initial experiments were
performed in the presence of \( 10^{-7} \) mol.\( l^{-1} \) phenoxyben-
zamine and relaxation responses studied after induced
tension with 5-HT. However, it was found that there
were no differences in results obtained if phenoxyben-
zamine was deleted from the tissue bath, and in the
few cerebral arteries studied with phenoxybenzamine
there were also no differences in the responses ob-
tained when this antagonist was omitted from the
bath. Therefore, all subsequent experiments were per-
formed in the absence of phenoxybenzamine.

Responses in cerebral arteries were studied in more
detail, as dilatation in the cerebral arterial bed is
postulated to be physiologically important in regula-
tion of cerebral blood flow. Many of the arteries were
tested to show that they were mechanically capable of
relaxing after inducing a resting tension by adding a
large concentration of either sodium nitroprusside or
sodium nitrite at the end of the experiment.

Results

1. Comparative Histology between Cerebral and Digital
Arteries

Figure 1 shows hemotoxylin and eosin stained
cross-sections of a human basilar artery (panel A-left)
and digital artery (panel B-right), obtained 15 h after
the death of a 58-year-old patient. Digital arteries
have a small lumen but thick muscular wall, sur-
rounded by much adventitial connective tissue.
Cerebral arteries tend to have larger lumens, but the
medial smooth muscle wall is much thinner. Advent-
titial connective tissue is sparse.
2. Contractile Responses of Cerebral and Digital Arteries

We have previously shown that, presumably because of the stability of vascular smooth muscle stored at 4°C, there is no relationship between time after death that digital arteries are removed (up to about 30 h) and the responses obtained in vitro. Nor is there any clear relationship between the responses and age, sex, cause of death or prior medication of the patient. This was also found to be the case for cerebral arteries, although there was definite deterioration in some of the vessels removed greater than 30 h after death.

In both the cerebral and digital arteries contractile responses were sought to both non-specific stimulation by KCl, BaCl₂ and to specific, receptor mediated stimulation by angiotensin, norepinephrine (NE), 5-HT and histamine. Responses were also obtained to prostaglandin F₂α. A typical example of a receptor mediated response (in this case to NE) in a digital artery is shown in figure 2. Concentration-dependent agonist responses could be obtained that were reproducible and durable for the length of an experiment and reversible upon washout. These were also a feature of the cerebral arterial responses. Some slight variability occurred in the responses between different arteries, therefore agonist responses were repeated in between 6 and 40 different arterial preparations.

Table 1 summarizes the mean pED₅₀ values in both the cerebral and digital arteries. The values of p show that none of these differences in sensitivity is statistically significant, hence suggesting that each agonist has the same sensitivity in both arterial beds.

Table 2 shows the mean agonist maximum response values (expressed as a percentage of KCl contracture; ± standard error of the mean) in both cerebral and digital arteries. The p values clearly show that none of the maximum responses is significantly different, except for NE where the maximum response is markedly greater in digital arteries.

NE is believed to be the sympathetic transmitter at the neuromuscular junction and likely to effect both cerebral and peripheral blood flow. Also, 5-HT has long been considered to have marked effects.
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physiologically on the cerebral arterial bed. Thus, the responses to NE and 5-HT in the 2 arterial beds were of particular interest and the full concentration effect curves in cerebral and digital arteries to both 5-HT and NE are shown separately in figure 3 left and 3 right respectively. These figures serve to emphasize that the maximum responses to 5-HT in both arterial beds were not significantly different, nor were the pED50 values significantly different. However, the concentration-effect curves to 5-HT in the 2 tissues were a slightly different shape so that at low concentrations of 5-HT the cerebral arteries gave significantly larger responses than the digital arteries (at 10^-4 mol.l^-1 5-HT, p < 0.02; 50 d.f.).

In contrast to this, while the maximum responses to NE were significantly different between cerebral and digital arteries (being larger in the digital arteries p < 0.001; 59 d.f.), the pED50 values were not significantly different in the two arterial beds (p > 0.05; 59 d.f.).

3. Effect of Cocaine on Arterial Responses

The fact that the major difference between cerebral and digital arteries was the diminished maximum response of the cerebral vessels to NE raised the question as to whether this may be due to different neuronal uptake mechanisms in the 2 tissues. This possibility was investigated by studying the effect of cocaine hydrochloride on responses to NE. It was found that cocaine minimally potentiated the effect of NE in some of the vessels at low concentrations of NE, but never enhanced maximum response to NE.

4. Relaxation Responses of Cerebral and Digital Arteries

Figure 4 shows a single example of the type of response to the relaxant agonists and, in this case, the response shown is that of histamine on a cerebral artery. Initial concentration-dependent relaxation responses have been obtained below the normal resting baseline tension. This was followed by a

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**FIGURE 3.** Concentration-effect curves for 5-hydroxytryptamine (left panel) and norepinephrine (right panel) in both digital and cerebral arteries.

**FIGURE 4.** Example of the concentration-effect relationship of a relaxant agonist (in this case histamine) in a cerebral artery. As can be seen, in higher concentrations histamine was a contractile agent.
concentration-dependent contractile response. Although this particular artery shows a good example of an initial relaxation response, it was found that less than half of the arteries tested responded to these agonists and, where relaxation responses were obtained, they were difficult to elicit and rather variable. The results were also not influenced by the method being used to elicit the relaxation responses, so the collective results are shown in table 3. Dopamine was the only agonist producing a relatively predictable response in a large percentage of cerebral arteries but, again, these responses were also generally fairly small. In the one digital artery where histamine produced a relaxant response, there was quite a marked initial relaxation. Of note was that relaxant beta-adrenergic receptors could not be demonstrated in either of the 2 arterial beds by the use of the beta-selective adrenergic agonist isoproterenol.

Discussion

Differing pharmacological and physiological responses of cerebral and peripheral vasculature are likely to be of clinical importance in man. Such differences might be receptor mediated or due to differing non-specific responses, so an agonist study has been carried out using human postmortem cerebral and digital arteries.

It has not been suggested in the literature that there is any difference in the non-receptor mediated responses of cerebral and peripheral arteries. This is confirmed by the findings of this study, where responses to KCl and BaCl₂ were qualitatively and quantitatively the same in both arterial beds. This was also the case with the response to prostaglandin F₂α and the receptor mediated responses to angiotensin and histamine. These findings thus suggest that there are no fundamental differences between these respective receptors in the two arterial beds. It also suggests that the method being used to assess the responses of these 2 tissues, despite obvious differences in their size and histological characteristics, is giving a true measure of their responses to the agonists being tested.

Of greater interest however, were the responses to NE and 5-HT. By the use of suitable antagonists, we have previously shown that NE and 5-HT stimulate separate receptors in digital arteries,¹⁰ and, by the same means, we have also demonstrated this to be the case in cerebral arteries. It is generally thought that cerebral arteries are considerably more sensitive to 5-HT than are the more peripheral arteries. This suggestion has been derived from experiments in many species, including cats, dogs, cattle, and humans. Our results do not confirm these previous suggestions, and this study is the first to directly compare detailed dose-response relationships of peripheral and cerebral vessels in humans. The maximum response to 5-HT and the median effective concentration of 5-HT, which define the pharmacological concentration-effect relationship, were not significantly different in the cerebral or peripheral arteries, thus suggesting that the tryptaminergic receptors are probably not pharmacologically different in these 2 vessels. The only significant difference between the 2 tissues was their responses to low concentrations of 5-HT. These low concentrations of 5-HT may, of course, be the most physiologically relevant, but it was also observed that the cerebral arteries tended to give slightly greater responses at the lowest concentrations of the other contractile agonists, so this effect is probably not specific to 5-HT.

It is also believed that the cerebral arterial alpha-adrenergic receptor is atypical, in that responses are generally extremely poor, and NE exhibits a very low efficacy in cerebral arteries.¹, ⁶, ¹², ¹⁶, ¹⁷ In fact, bovine cerebral arteries have been reported not to respond at all to alpha-receptor agonists such as NE.¹⁸

The findings in this study do confirm an altered response of the cerebral arteries to NE, in that the maximum response to that agonist is markedly reduced. However, the finding that the \( p_{ED_{50}} \) for NE is the same in cerebral and digital arteries suggests that the alpha-adrenergic receptors in the cerebral arterial bed are probably not pharmacologically atypical. Rather, it suggests that the differences in efficacy of NE in digital and cerebral arteries may be explained by a smaller population of alpha-receptors in the cerebral vessels.

It is possible that the differing efficacy of NE demonstrated in this study might be explained by differences in neuronal and extraneuronal uptake of NE in the 2 tissues. However, this would be expected

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Table 3  Summary of Results Using Relaxant Agonists on Both Cerebral and Digital Arteries. Mean Maximum Relaxation Response (as a % of KCl; ± Standard Error of Mean) is Shown Only for Cerebral Arteries

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Cerebral n</th>
<th>Number of arteries giving relaxant response</th>
<th>Mean maximum* relaxant response (as % KCl) ± SME</th>
<th>Digital n</th>
<th>Number of arteries giving relaxant response</th>
<th>Maximum relaxant response (as % KCl) of responding vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol</td>
<td>33</td>
<td>15</td>
<td>6.26 (± 1.50)</td>
<td>5</td>
<td>1</td>
<td>4.4</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>14</td>
<td>6</td>
<td>12.80 (± 5.00)</td>
<td>5</td>
<td>1</td>
<td>10.4</td>
</tr>
<tr>
<td>Histamine</td>
<td>17</td>
<td>7</td>
<td>23.31 (± 11.29)</td>
<td>5</td>
<td>1</td>
<td>67.7</td>
</tr>
<tr>
<td>Dopamine</td>
<td>14</td>
<td>11</td>
<td>16.14 (± 6.99)</td>
<td>5</td>
<td>1</td>
<td>16.3</td>
</tr>
</tbody>
</table>

n = number of different arteries on which the respective agonist was tested. *Mean only of those vessels which gave a relaxant response.
to alter the \( pED_{50} \) rather than the maximum response to NE, as any uptake processes should be saturated at high concentrations of NE and the maximum response should still be reached. Moreover, the observation that cocaine has only minimal or no potentiating effect on the responses to NE must strongly suggest that the differences in the efficacy of NE in the 2 tissues is not due to differences in neuronal uptake.

Dilatation of arterial beds is of equal importance to constriction in the regulation of blood flow, so we sought relaxation responses to the agonists isoprotrorenol, acetylcholine, histamine and dopamine. Although relaxation responses are difficult to demonstrate \textit{in vitro} in tissues which are maximally relaxed and thus must be given a resting tone by the addition of a contractile agonist, we were able to demonstrate that our vessels were capable of relaxing by the addition of non-specific vasodilating drugs such as sodium nitrite or sodium nitroprusside. Yet we were not able to demonstrate consistent relaxation responses to any of the receptor mediated relaxant agonists. Beta-adrenergic receptor mediated vascular relaxation, especially in cerebral vessels, has been demonstrated in other species, but in a total of 33 vessels in which such relaxation was sought, less than half relaxed at all, and where responses were obtained in all cases it was only minimal. Relaxation responses to dopamine, although never very great, were the most consistently observed, and responses to histamine were the most variable, with occasional vessels giving good relaxation responses. This study therefore, suggests that beta-adrenergic and cholinergic receptors are of little functional significance in either vascular bed, that dopaminergic receptors may well be present in small numbers, particularly in the cerebral vessels, and relaxant histamine receptors are variably present in both vascular beds.

Care must always be exercised in extrapolating results obtained on isolated tissues to the physiological situation in intact man. However, the results from this agonist study suggest that the cerebral and peripheral vascular beds in man are probably not as pharmacologically different as has been previously thought. The only major difference between the 2 vascular beds would appear to be the lesser importance of noradrenergic mechanisms in the regulation of human cerebral blood flow.

Acknowledgment

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References


Erratum

In Levinthal et al. (Stroke 10, 371–375, July-August, 1979), Page 374, change reference 7 to read:

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