Rapid Serial Angiography in the Investigation of the Pharmacology of Brain Circulation in the Dog

By Giancarlo Canossi, M.D., Brunello Bruncilli, M.D., Nicola Cortesi, M.D., and Amilcare Carpi, M.D.

The important contribution of the study of the cerebral circulation to the physiology and pharmacology of the central nervous system has led to the elaboration of new methods for a more extensive investigation of the reactions of the intracranial circulatory area. Each technique has certain advantages and limitations: the direct methods, hemodynamic in principle, allow a continuous registration of the variations in cerebral circulation, but do not usually provide an absolute measure of flow; on the other hand, the indirect methods, and in particular, the technique of Kety and Schmidt based on the Fick principle, provide an absolute measure but not a continuous registration of the cerebral blood flow.

The principal advantage of having both types of methods available is that the two are complementary, one providing certain information not easily obtainable by the other. In fact, the most convincing data concerning the physiology and pharmacology of cerebral circulation are those which support the same conclusions but are obtained with these two different methods. In addition, since one of the principal aims of experimental research in this field is to provide a comparison with the results obtained in man, it is evident that the use, in the experimental animal, of techniques devised for the clinical study of cerebral circulation facilitates this comparison.

The application, in the study of cerebral circulation, of the serio-angiographic technique, which has been utilized in the study of other vascular areas, has allowed the determination of cerebral circulation times in man. Tönnis and Schiefer obtained comparable results using this method and the one of Kety and Schmidt.

In our previous work, the cerebral venous pressure of the dog has been taken as an indication of the circulatory condition of the brain, since it is a relatively simple method, can be carried out with a minimum of trauma, and provides for a continuous registration of alterations in circulation. To provide a further evaluation of this technique, in the present study, the reactions of the cerebral circulation to pharmacological agents were examined in parallel by rapid serio-angiography and by the registration of the cerebral venous pressure.

Methods

The experiments were carried out on dogs weighing 12 to 25 Kg. under chloralose anesthesia (80 to 200 mg./Kg., I.V.): in the preliminary observations, the animals were allowed to breathe spontaneously; in the procedure finally adopted, the dogs were treated with gallamine (4 mg./Kg., I.V.) and were maintained under artificial respiration at a rate of 30 to 35 minutes with the amplitude set in such a manner as to maintain the arterial and the intracranial venous pressures at the same levels existing before the administration of gallamine. This procedure enabled changes in cerebral vascular tone depending on variations of gas content of arterial blood to be held to a minimum.

ANGIOGRAPHIC METHOD

In a short series of preliminary investigations, the contrast medium was injected into the vertebral artery through a polyvinyl catheter introduced into that artery at a point just before it enters its vertebral canal. In the final experiments, the carotid route was used; a thin catheter (inner diameter, 0.5 mm.) was introduced into the common carotid artery through the superior thyroid artery; special care was taken to prevent the tip of the catheter from going beyond the origin of the internal carotid artery. Regarding the dose of the contrast medium, a good visualization of the intracranial circulation was obtained by injecting 4 ml. of compounds commonly employed in the clinical investigation (sodium acetrizoate, 45 per cent;
FIGURE 1
Systemic and cerebral vascular reactions to the intracarotid injection of 4 ml. of sodium diacetrizoate (50 per cent) in the chloralosed dog. Male dog: 25 Kg. (10/26/59), chloralose (100 mg./Kg. I.V.). (A) Femoral arterial pressure (mm. Hg); (V) intracranial venous pressure (cm. H$_2$O). The serio-angiographic record was taken in the period indicated by the shaded column.

sodium diacetrizoate, 50 per cent); the injection was performed under manual pressure as soon as possible; usually the whole dose was injected in about one second.

The serio-angiographic photos were taken in lateral projection by means of the Sanchez-Perez serio-graph with the following parameters: intensity 200 ma., 58 to 60 kv.; exposure 0.08 second; focus film distance 100 cm. Since the intracranial circulation time of the dog is rather brief under normal conditions, the complete course of the contrast medium along the cerebral vascular tree could be demonstrated in a series of 8 angiograms taken at intervals of 0.5 to 0.8 second; series of 12 angiograms were only recorded after the administration of drugs which caused marked lengthening of the cerebral circulation time. The serio-angiographic record was initiated at the moment corresponding to the beginning of the injection of the contrast medium. In this way, in the first angiogram it was possible to visualize the internal and the external carotid arteries.

The cerebral circulation time was calculated in two ways: first, by evaluating the time lapse between the beginning of the visualization of the internal carotid artery and of the visualization of the ascending cerebral veins (initial circulation time); secondly, by calculating the interval between the end of visualization of both vessels (final circulation time). The beginning and the end of visualization of these vessels do not always correspond to a definite angiogram; in these cases, following the principles already applied in the clinical investigation, these times have been fixed at the middle of the interval between two consecutive angiograms.

HEMODYNAMIC METHODS
The systemic and the cerebral circulatory conditions were followed by registering the arterial pressure from the femoral artery by means of a mercury manometer and the intracranial venous pressure by means of a water manometer connected with a polyvinyl catheter introduced into the external jugular vein and manipulated, by way of the internal jugular vein, until the tip was as close as possible to the superior cerebral vein. A continuous tracing of the arterial pressure was taken photographically, whereas the values of the intracranial venous pressure were read every 10 seconds.

DRUGS
Strychnine (0.5 mg./Kg.) and ergotamine tartrate (10 /xg./Kg.), which exert clear cerebral vascular effects, were administered intravenously; the motor component of the strychnine convulsions did not appear since the animals were always treated with gallamine (4 mg./Kg.) and maintained under artificial respiration.

Results
PRELIMINARY OBSERVATIONS
The aim of the first experiments was to establish the most suitable conditions for satisfactory visualization of the intracranial vessels. It was thus observed that the administration of the contrast medium by way of the vertebral artery always provoked marked syncopeal phenomena which very often resulted in the death of the animal. Therefore, the carotid route was adopted. Although the injection of the contrast medium by this route often provoked a marked arterial hypotension, due to a transient cardiac arrest, followed by an increase in the intracranial venous pressure (fig. 1), it was observed that the administration of either atropine (0.5 mg./Kg., I.V.) or high doses of gallamine (4 mg./Kg., I.V., fig. 2) completely prevented the appearance of this bradycardia and of the subsequent hypo-
Absence of vascular reactions to the intracarotid injection of 4 ml. of sodium diatrizoate (50 per cent) in the chloralosed dog treated with gallamine. Female dog: 12 Kg. (10/30/59), chloralose (100 mg./Kg., I.V.), gallamine (4 mg./Kg., I.V.), artificial respiration. Recordings as in figure 1. Under these conditions, the seriosangiographic record demonstrated a good progression of the contrast medium along the arterial and then the venous section of the intracranial vascular tree. Control experiments showed that repeated administrations of the medium up to four times in the same animal showed no significant variations in circulation time when the intracranial venous pressure and the arterial blood pressure, at the time of each injection, were comparable. That portion of the contrast medium which entered the extracranial vessels allowed a comparison of the reactivity of the intra- and extracranial circulation. Thus, the procedure finally adopted involved the injection of the contrast medium by the carotid route in animals already in neuromuscular paralysis and under artificial respiration.

MORPHOLOGY OF THE CEREBRAL CIRCULATION

The angiographic anatomy of the cerebral circulation of the dog has been described recently, and therefore only the morphological data on which the seriosangiographic evaluation of the intracranial circulation time is based will be considered here. The internal carotid artery and the ascending cerebral veins have been chosen as the starting and the end point of cerebral circulation. The first can be recognized easily by its funnel-shaped origin from the common carotid artery and by its course crossing the occipital and the great auricular arteries and directed toward the intense opacity of the basal bones of the skull (fig. 3). The ascending cerebral veins can be
detected in the parietal region and present an ascending course leading to the superior sagittal sinus (fig. 4). These vessels collect the blood coming only from the intracranial vessels, whereas other veins, such as the cerebrofacialis and the internal maxillary, collect also a significant portion of extracranial blood. The ascending cerebral veins have been chosen as the end point of the intracranial circulation because they were visualized, under good cerebral circulatory conditions, in all but one experiment (dog 48); in this case, the extremely high rate of the brain circulation probably prevented the brief phase of visualization of these vessels to be fixed in any one angiogram of the series. Numerous extra-

CEREbral CIRCULATION TIME IN THE ANESTHETIZED ANIMAL

The results of a group of experiments are shown in the table 1, which gives the data obtained: (a) under basal conditions (chloralosed dog, treated with gallamine and under artificial respiration); (b) during the cerebral vasodilatation induced by strychnine (0.5 mg./Kg., I.V.); and (c) during a phase of cerebral vasoconstriction after the administration of ergotamine (10 mg./Kg., I.V.)

A reliable evaluation of the circulation time can be obtained when the results from dogs under basal conditions are compared with those observed in the animal treated with strychnine. In fact, strychnine is able to restore a normal circulatory condition in the brain in which the circulation has been depressed by anesthesia or surgical trauma and is lacking the physiological and pharmacological reactivity which, in the animal as well in man, indicates a normal hemodynamic picture.

When data obtained with dogs under basal conditions are grouped with those from dogs pretreated with strychnine, the initial circulation time (see Methods) had a mean value of 2.3 ± 0.33 seconds with fiducial limits (P < 0.05) of 1.46 and 3.14 seconds (seven determinations, five animals); the mean value of the final circulation time was 3.2 ± 0.50 with fiducial limits (P < 0.05) of 1.92 and 4.48 seconds (seven determinations, five animals). In the same experiments, the intracranial venous pressure had a mean value of 13 ± 2.10 cm. H_{2}O with fiducial limits (P < 0.05) of 8.21 and 18.99 cm. H_{2}O (seven determinations, five animals): these limits correspond to the minimal and maximal levels of the intracranial venous pressure obtained in a wide series of observations. Since the absolute value of the intracranial venous pres-
TABLE 1
Cerebral Circulation Time in Dogs Anesthetized with Chloralose

<table>
<thead>
<tr>
<th>Dog</th>
<th>Treatment</th>
<th>Arterial pressure (mm. Hg)</th>
<th>Intracranial venous pressure (cm. Hg)</th>
<th>Beginning and end of visualization Internal carotid artery (seconds)</th>
<th>Ascending cerebral veins (seconds)</th>
<th>Circulation time (seconds)</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>Ergotamine</td>
<td>130</td>
<td>14.5</td>
<td>0 to 0.8</td>
<td>1.6 to 4.0</td>
<td>1.6</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Strychnine</td>
<td>140</td>
<td>12.0</td>
<td>0 to 0.8</td>
<td>2.4 to 4.8</td>
<td>2.4</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Ergotamine</td>
<td>145</td>
<td>9.0</td>
<td>0 to 0.8</td>
<td>2.4 to 6.4</td>
<td>2.4</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>Control</td>
<td>105</td>
<td>9.0</td>
<td>0 to 0.8</td>
<td>3.6 to 5.2</td>
<td>3.6</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strychnine</td>
<td>155</td>
<td>22.0</td>
<td>0 to 0.4</td>
<td>2.0 to 5.4</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ergotamine</td>
<td>155</td>
<td>13.5</td>
<td>0 to 0.8</td>
<td>1.6 to 3.2</td>
<td>1.6</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Control</td>
<td>115</td>
<td>8.0</td>
<td>0 to 0.4</td>
<td>2.8 to 5.6</td>
<td>2.8</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strychnine</td>
<td>160</td>
<td>20.0</td>
<td>0 to 0.5</td>
<td>1.0 to 2.0</td>
<td>1.0</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ergotamine</td>
<td>150</td>
<td>14.5</td>
<td>0 to 0.4</td>
<td>1.6 to 2.8</td>
<td>1.6</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Control</td>
<td>110</td>
<td>9.5</td>
<td>0 to 0.5</td>
<td>2.0 to 3.5</td>
<td>2.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strychnine</td>
<td>135</td>
<td>18.5</td>
<td>0 to 0.25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ergotamine</td>
<td>115</td>
<td>14.0</td>
<td>0 to 1.0</td>
<td>1.0 to 2.5</td>
<td>1.0</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ergotamine</td>
<td>120</td>
<td>7.5</td>
<td>0 to 0.75</td>
<td>1.5 to 3.0</td>
<td>1.5</td>
<td>2.25</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>Control</td>
<td>100</td>
<td>15.0</td>
<td>0 to 0.5</td>
<td>3.0 to 3.5</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ergotamine</td>
<td>170</td>
<td>13.0</td>
<td>0 to 1.0</td>
<td>3.5 to 5.0</td>
<td>3.5</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>Ergotamine</td>
<td>100</td>
<td>15.5</td>
<td>0 to 1.0</td>
<td>1.75 to 3.0</td>
<td>1.75</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

Sure seems to be a reliable indication of the level of the cerebral blood flow, the relationship between the intracranial venous pressure and the cerebral circulation time has been analyzed. A statistically significant inverse correlation between the final circulation time and the cerebral venous pressure has been found (fig. 5A); this correlation was not significant when the initial circulation time was considered (fig. 6A).

ACTION OF ERGOTAMINE

To evaluate the usefulness of the serio-angiographic method in the pharmacological study of brain circulation, the intracranial circulation time has been determined in the animal treated with ergotamine. Previous work had demonstrated that this drug, administered intravenously in a dose of 10 µg./Kg., provokes a long-lasting fall in the intracranial venous pressure, which results from a vasocstriction of the cerebral vessels. This action is more evident in the dog in which a marked cerebral vasodilatation has been induced previously by strychnine. The experiments reported here indicate that the fall in the intracranial venous pressure was accompanied by a rise of the cerebral circulation time. Considering the complete series of data obtained under basal conditions, after the administration of strychnine and after the injection of ergotamine, the good inverse correlation existing between the values of the intracranial venous pressure and the final circulation time is confirmed (fig. 5B), whereas the poor correlation between the cerebral venous pressure and the initial circulation time is still more evident (fig. 6B).

In addition to a consideration of the dynamic data, it is worth referring to some morphological modifications of the cephalic angiogram induced by ergotamine. In most cases after the administration of ergotamine the diameters of the extracranial arteries (superficial temporal, great auricular, inferior alveolar artery) were reduced, whereas the intracranial vessels were seen more clearly as if a greater portion of the contrast medium had now been diverted to the intracranial district. The dynamic effects of ergotamine and some of these morphological modifications induced by the drug are shown in figures 7 and 8.

Discussion

CEREBRAL CIRCULATION TIME

The observed values of the cerebral circulation time have shown a variability which appears greater than that obtained by Greitz.
Correlation between the observed values of the intracranial venous pressure (abscissa, cm. H$_2$O) and those of the final cerebral circulation time (ordinate, seconds): (A) data obtained under basal conditions and after strychnine; (B) pooled data; (O) dogs under basal conditions; (△) dogs treated with ergotamine (10 μg./Kg., I.V.); (φ) dogs treated with strychnine (0.5 mg./Kg., I.V.). The data obtained in the same dog are connected by lines as follows: (——) dog 29; (----) dog 41; (-----) dog 42; (—) dog 43; (——) dog 45; (——) regression line.

in man (4.13 ± 0.13 seconds). In the experiments referred to here, many factors are responsible for this marked variability; the level of the arterial pressure, varying between 100 and 170 mm. Hg, and the size of animals (12 to 25 Kg.) must certainly play a role. However, there is a more important factor to be considered, namely, the level of the cerebral blood flow. This is clearly demonstrated by the significant inverse correlation existing between the cerebral circulation time and the value of the intracranial venous pressure which is higher the greater the cerebral blood flow. The correlation, which is statistically significant in the group of observations under basal condition and after strychnine, as well as when all the data are pooled, is valid only if the values of the final circulation time are considered. Probably the poor correlation existing between the intracranial venous pressure and the initial circulation time is due to the fact that this time, being shorter than the final circulation time, cannot be evaluated with sufficient accuracy on a serigraphic record taken at a rate of one angiogram every 0.5 second. It is thus possible that, under these conditions, the modific
Effect of ergotamine on the angiographic cerebral circulation time. Female dog: 14 Kg. (no. 43, 10/31/59), chloralose (100 mg./Kg., I.V.), gallamine (4 mg./Kg., I.V.), artificial respiration. Rapid serio-angiograms taken after administration of strychnine (0.5 mg./Kg., I.V.) and before the injection of ergotamine. Intracranial venous pressure 20 cm. H$_2$O; rate of serigraphic record: 1 angiogram every 0.5 second. (1) Beginning of visualization of internal carotid artery: first angiogram (0 seconds); (2) end of visualization of internal carotid artery: second angiogram (0.5 second); (3) beginning of visualization of cerebral ascending veins: third angiogram (1.0 second); (4) end of visualization of cerebral ascending veins: fifth angiogram (2.0 seconds); (5) initial circulation time: 1.0 seconds; (6) final circulation time: 1.5 seconds.
FIGURE 8

Effect of ergotamine on the angiographic cerebral circulation time. Female dog: 14 Kg. (no. 43, 10/31/59), chloralose (100 mg./Kg., I.V.), gallamine (4 mg./Kg., I.V.), artificial respiration. Rapid serio-angiogram taken after the injection of ergotamine (10 µg./Kg., I.V.). Intracranial venous pressure 14.5 cm. H₂O; rate of serio-angiographic record: 1 angiogram every 0.8 second. (1) Beginning of visualization of internal carotid artery: first angiogram (0 second); (2) end of visualization of internal carotid artery: between the first and the second angiogram (0.4 second); (3) beginning of visualization of cerebral ascending veins: third angiogram (1.6 seconds); (4) end of visualization of cerebral ascending veins: between the fourth and the fifth angiogram (2.8 second); (5) initial circulation time: 1.6 seconds; (6) final circulation time: 2.4 seconds.
CEREBRAL ANGIOGRAPHY

This effect does not negate previous conclusions on the cerebral vasoconstrictor effect of ergotamine; it merely indicates, when circulation time is also taken into account, an increase in concentration of radiopaque material in the intracranial vessels.

On the whole, these results indicate that ergotamine exerts a cephalic vasoconstrictor action which is more effective on the extracranial vessels; as a consequence, under normal conditions, the drug provokes a diversion of the carotid blood to the intracranial circulation and a simultaneous reduction of the cerebral blood flow, as demonstrated by the lengthening of the cerebral circulation time and by the fall in the intracranial venous pressure. On the other hand, it may be suggested that the diversion from the extra- to the intracranial circulation may be involved in the mechanism underlying the disappearance of the cerebral vasoconstrictor response to ergotamine in dogs with low levels of brain circulation. Under these conditions ergotamine, as well as other vasoconstrictor agents,7, 8 no longer reduces the cerebral blood flow, whereas the vasoconstrictor response to ergotamine is still present in the extracranial vessels. Lack of cerebral vasoconstrictor effects has also been seen in man under conditions of reduced cerebral blood flow10 and in severe hypocapnia associated with metabolic acidosis in diabetic coma.20

The serio-angiographic observations reported here illustrate, therefore, new aspects concerning the lability of the cerebral vasoconstrictor reactions2 and show the usefulness of the rapid serio-angiographic method not only in the pharmacological study of brain circulation, but also in the analysis of problems regarding the relationship between the extra- and the intracranial circulation.

Summary

The values of cerebral circulation time were determined by means of rapid serial angiography in the chloralosed dog under control conditions, after administration of strychnine and after injection of ergotamine. These re-

PHARMACOLOGICAL OBSERVATIONS

The serio-angiographic data confirm previous results obtained by means of the registration of the intracranial venous pressure and show new aspects of the cephalic vascular reactivity.

Strychnine markedly reduces the cerebral circulation time; this demonstrates, on a serio-angiographic basis, the increase in cerebral blood flow which can be not only dependent upon the simultaneous rise in the arterial pressure, but also upon the cerebral vasodilatation usually obtained with the administration of strychnine. This vasodilatation, according to combined hemodynamic and electroencephalographic observations, appears to be connected with the enhancement of the functional activity of the brain induced by the drug.10

Ergotamine clearly prolongs the angiographic circulation time, thus giving new evidence of the vasoconstrictor action exerted by this drug on the brain circulation of the chloralosed dog having normal or high levels of cerebral blood flow.

Regarding the extracranial circulation, the narrowing of the arteries in this region induced by ergotamine indicates an extracranial vasoconstrictor action in agreement with the clinical observations of Wolff15 and of Bovet and Gatti.16

Also the more intensive visualization of the intracranial vessels often observed after ergotamine is strongly indicative of an extracranial vasoconstriction since this is able to induce a diversion of the carotid blood from the extra- to the intracranial vascular area.17, 18

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suits were compared with those obtained by the simultaneous registration of the intracranial venous pressure. Confirming previous observations made by means of this latter technique, the angiographic results showed that strychnine reduces the cerebral circulation time by provoking an increase in cerebral blood flow, whereas ergotamine causes a cerebral vasoconstriction, thereby increasing intracranial circulation time. Moreover, a statistically significant inverse correlation exists between the values of the intracranial pressure and the cerebral circulation time. New evidence of the relationship between the extracerebral and the intracranial circulation was obtained by the angiographic observations; these latter data are discussed in relation with the problem of the lability of cerebral vasoconstrictor reactions.

Acknowledgment
The authors wish to thank Drs. Mirko Dardari and Costantino Pasquinelli for their assistance and the helpful discussion during the course of this work.

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Circulation Research, Volume X, March 1962
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doi: 10.1161/01.RES.10.3.259

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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