Animal Model of TIA: An Experimental Study With Intracarotid ADP Infusion in Rabbits

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Abstract:

Adenosine diphosphate (8 mg per minute for five minutes) was infused into the carotid artery of 63 rabbits. The effects were twofold: systemic hypotension and platelet aggregation in the cerebral circulation. As a consequence of the last effect, platelet emboli were produced which occluded cerebral arteries in a number and size sufficient to cause cerebral ischemia. Areas of focal ischemia were observed through a cranial window, and documented with antipyrine autoradiography.

Platelet thrombi were almost entirely transient, being fragmented and removed within a very short time of cessation of ADP infusion. Consequently, no permanent tissue damage ensued. This experimental model approaches the spontaneous transient ischemic attacks (TIAs) in man, demonstrating that these can be caused by pure platelet emboli.

A high cholesterol diet administered for two months prior to ADP infusion did not enhance the effect of the procedure or make the platelet aggregation and the following ischemia longer in duration or more severe.

Additional Key Words

- cerebral circulation
- platelet aggregation
- antipyrine autoradiography
- platelet emboli
- hypercholesterolemia

Because of the multiplicity of circumstances associated with human cerebral infarction, no animal model has yet been devised which closely resembles the condition in man. Some models may subject the entire animal to vascular insufficiency, while others involve various methods which interfere with local perfusion to the brain by vascular ligation or by artificial increases of intracranial pressure.

External occlusion of the carotid arteries, possibly combined with systemic hypotension or replacement of the atmospheric air by nitrogen or nitrous oxide, has been used in several studies, as well as occlusion of intracranial branches such as the anterior cerebral, posterior cerebral and, more frequently, the middle cerebral arteries.

A quite different approach, aimed at obtaining embolic occlusion of small intracranial branches of the carotid artery without subjecting the animal to either intracranial operation or direct surgical manipulation of the arteries, has been devised by intracarotid injections of microspheres in several animal species. In the present model, we produced embolization by inducing autologous platelet microemboli by means of selective ADP infusion in the carotid artery of rabbits through a collateral branch in the neck.

Adenosine diphosphate induces platelet aggregation both in vitro and in vivo. The in vitro effect on plasma-enriched human platelets is observed from a concentration of $5 \times 10^{-7}$ M up to a concentration of $2 \times 10^{-5}$ M. In vivo, platelet aggregation and formation of platelet emboli with secondary ischemia were observed in swine and rabbits at a concentration of $4 \times 10^{-4}$ M. Both effects, however, are transient, since platelets disaggregate within 20 minutes, with the exception of some permanent effects on tissues such as...
those observed by Jørgensen et al.12, 13 The authors apparently have been able to produce actual infarction in the myocardium and kidney with selective ADP infusion in the afferent arteries to these organs.

In such a model, pathophysiological effects upon the cerebral circulation might be quite similar to those produced by spontaneous platelet emboli in human atherosclerotic subjects. Thus, this animal model is described here and proposed for experimental studies of cerebral transient ischemic attacks (TIAs).

Methods

This study used 63 young male rabbits, each weighing approximately 2 kg. For two months, 22 rabbits were fed a cholesterol-rich diet of 1 gm per day, while 41 rabbits received a standard diet.

Anesthesia was induced by an intravenous injection of pentothal (30 mg per kilogram) and maintained with a mixture of O2 and N2O of 40% to 60%. The animals were tracheotomized and cannulated, and their respiration was stimulated artificially with the use of a Harvard respirator. The end-expiratory CO2 was measured by means of a Godart Capnograph (Paco, being maintained between 88 and 34 mm Hg), while the blood pressure was measured with a Statham transducer through a catheter previously inserted in the femoral artery. The rectal temperature also was monitored and kept between 36° and 38°C. These measurements were registered with a Grass polygraph throughout the experiment. The retrograde cannulation of the cerebral circulation might be quite similar to those described here and proposed for experimental studies of cerebral transient ischemic attacks (TIAs).

This solution, thus prepared and maintained at a constant temperature of 37°C, was infused for five minutes at the rate of 1 ml per minute (8 mg of ADP in one minute). The perfusate, diluted in the carotid blood, reached a final concentration of ADP equal to 10−3 M. In 18 animals, a catheter placed in the internal jugular vein permitted sampling of mixed cerebral venous blood for platelet counts. Thirty-six rabbits had a craniotomy performed to expose through a cranial window an area of the cerebral cortex to permit visualization of the pial vessels with a Zeiss dissection microscope. In those animals, 0.5 ml of Evans blue was injected through the lingual artery and the ligature of all the major branches of the common carotid artery were performed without interruption of blood flow in the internal carotid artery.

Forty milligrams of ADP* were dissolved in 5 ml of Ringer's solution buffered at pH 7.4 and kept at a temperature of 37°C for 15 minutes before use.

Epinephrine (1 × 10−6 mg) was added to the solution. This solution, thus prepared and maintained at a constant temperature of 37°C, was infused for five minutes at the rate of 1 ml per minute (8 mg of ADP in one minute). The perfusate, diluted in the carotid blood, reached a final concentration of ADP equal to 10−3 M. In 18 animals, a catheter placed in the internal jugular vein permitted sampling of mixed cerebral venous blood for platelet counts. Thirty-six rabbits had a craniotomy performed to expose through a cranial window an area of the cerebral cortex to permit visualization of the pial vessels with a Zeiss dissection microscope. In those animals, 0.5 ml of Evans blue was injected through the lingual artery to verify that the ADP infusion had reached the brain. After two such ADP infusions, separated by a 30-minute interval, the animals were killed and the vessels (circle of Willis and pial branches) were explored.

Five more rabbits (standard diet) were injected with 750 mc of radioactive antipyrine (I131 or C14) in 40 seconds, at a constant rate, in the femoral vein. The antipyrine infusion took place from four minutes to four minutes and 40 seconds during ADP infusion, and was used to study the blood flow distribution in the brain. These animals were then killed immediately with KCl. The brain, removed and frozen in a mixture of acetone and dry CO2, was cut in 20 micron slices at −20°C for autoradiography. In the C14 rabbits, arterial blood samples were collected during isotopic infusion.14

For the long-term study, the EEG was recorded in four rabbits (cholesterol diet) with chronic electrodes implanted in the cranial bone. These animals were killed ten days after the infusion of ADP in order to estimate histologically the possible parenchymal lesions and the persistence of arterial occlusion. Their brains, fixed in formalin and mounted in paraffin, were sliced and stained with hematoxylin and eosin.

Results

Platelet Count and Cranial Window Observations

ADP has a constant and significant effect on systemic blood pressure, decreasing it by 50% (to 54 ± 12.3 mm Hg) during the first minute of infusion (table 1). This effect occurs in spite of the fact that ADP is selectively infused in the carotid artery, with its concentration in the systemic arterial blood reaching a maximum level of 5 × 10−8 M. The pressure drop is gradually reduced during the five minutes of infusion so that when emboli are observed in the pial arteries, pressure reduction is less than that indicated in table 1. As shown, pressure is very rapidly restored upon cessation of ADP infusion.

The change in platelet count in the mixed cerebral venous blood of the internal jugular vein is similar in time to that of the blood pressure, with subsequent restoration during the infusion of ADP and a return to normal shortly thereafter (fig. 1).

The time pattern of changes observed in the pial vessels through the cranial window is somewhat different. In all rabbits, platelet emboli were clearly seen traversing the cortical branches and stopping at arteriolar bifurcations. The first emboli are seen only two minutes after initiating the infusion, and reach maximum density by the third or fourth minute. Multiple emboli accumulate, occluding collateral branches, thus producing cortical pallor and focal ischemia.

On the other hand, even before cessation of the infusion, one could notice fragmentation of embolic material removed from the cortical branches, those branches being almost entirely reperfused one minute after cessation of infusion. This latter phenomenon was enhanced by the resumption of normal blood pressure.

In one-third of the animals, however, small embolic occlusions persisted, although scanty and scattered, so that all parts of the visible cortex were normally perfused through collateral branches. This lasted up to 30 minutes.

At this time, we repeated a second ADP infusion

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td><strong>SAP During ADP Infusion</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
</tr>
<tr>
<td>SD</td>
</tr>
</tbody>
</table>

SAP = systemic arterial pressure.
and noticed that the platelet emboli and consequent transient cerebral ischemia were reproduced. Although no attempt to quantify this effect was made in this study, it appears that there is no systematic difference between the 18 cholesterol-fed and the 18 standard-diet rabbits studied with the cranial window.

**ANTIPYRINE STUDIES**

$^{131}$I antipyrine, when infused in a systemic vein for 40 seconds at or slightly later than the time of maximal embolization, is a topographically precise method to detect whether or not the occlusions produce tissue ischemia. Antipyrine is distributed as a function of blood flow and, even in the presence of embolization in small arteries, its diffusion in the tissue would not be affected in the presence of sufficient collateral branches.

In fact, we were able to document ischemic areas by this technique in all three rabbits thus studied. Quantitative $^{14}$C-antipyrine studies in two additional rabbits gave average regional cerebral blood flow data reported in table 2.

The ischemic areas at different stages are observed in figures 2 and 3. Some are areas of subtotal ischemia, round and obviously peri-arteriolar when located in deep portions of the brain (caudate nucleus, subcortical white thalamus) or cuneate when in the superficial gray matter. Others are areas of less complete and already partially recovered ischemia and may even show reactive hyperemia at their periphery. The extension of the observed ischemic areas varied from 2.2% to 18.7% of the middle cerebral territory.

We consider the present experiments as being the first objective visualization of transient focal ischemia in the brain.

**OBSERVATIONS ON FUNCTIONAL, CLINICAL AND HISTOLOGICAL CONSEQUENCES OF ISCHEMIA PRODUCED BY PLATELET EMBOLI**

In the acute stage, when the animals are lightly anesthetized, an immobilized clinical effect, if present, cannot be seen. Functional consequences of ischemia on the CNS, however, are documented by EEG changes, which were studied in the four animals subsequently used for long-term effect studies.

Unilateral reduction of amplitude and low frequencies recorded in those animals from scalp electrodes on the side of ADP infusion indicated that in this group focal ischemia was produced (fig. 4). In

<table>
<thead>
<tr>
<th>Structure</th>
<th>Cerebral Blood Flow (ml/100 gm/min)</th>
<th>Left</th>
<th>Right</th>
<th>$\Delta$ % change</th>
</tr>
</thead>
<tbody>
<tr>
<td>White matter</td>
<td></td>
<td>23.5</td>
<td>14.8</td>
<td>-37.0</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td></td>
<td>76.7</td>
<td>39.2</td>
<td>-48.9</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td></td>
<td>68.0</td>
<td>39.5</td>
<td>-41.9</td>
</tr>
<tr>
<td>Cortex</td>
<td></td>
<td>87.2</td>
<td>46.5</td>
<td>-46.6</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td>63.7</td>
<td>52.8</td>
<td>-19.6</td>
</tr>
</tbody>
</table>

*Mean.

Rabbit No. 73 (same as fig. 2): section at the level of the basal ganglia. Several totally or partially ischemic areas in the right MCA territory with perifocal reactive hyperemia.
these animals, which were prepared for chronic studies, the effect of ADP infusion was not checked more directly with the cranial window or with antipyrine autoradiography. EEG changes also were transient, however, as were the vascular changes directly observed in the previous group.

These animals recovered from anesthesia after a repeated ADP infusion, and no clinical damage was apparent in the following days. Ten days later they were killed. No vascular occlusion was observed on systematic examination of the intracerebral and extracerebral arteries, nor in smaller vessels at histology. Similarly, no cellular damage indicative of persistent ischemic alterations was noted in the many sections from the four animals examined. All evidence thus points to the production of a reproducible but transient cerebral ischemia by selective ADP infusion in the carotid artery.

LACK OF ENHANCEMENT OF ADP EFFECTS IN HYPERCHOLESTEROLEMIC RABBITS

In view of the possible platelet alterations found in experimental hypercholesterolemia, it was our aim to test whether, after a month of high cholesterol diet, ADP infusion produced a stabilized vascular occlusion with a more extensive and permanent ischemic tissue damage.

Changes of the pial circulation, observed in 17 animals through a cranial window after ADP infusion, were neither more consistent nor of longer duration than those noticed in normally fed animals.

It might be coincidental that among the animals killed in the acute phase, 30 minutes after the last ADP infusion, occasional emboli were still present in small arterial branches of a major branch (or branches) of the middle cerebral artery of the cholesterol-fed group. Sometimes small fibrin-like clumps were present (fig. 5). Permanent brain infarcts could be produced by this technique if the emboli remained for sufficient duration. This apparently was not observed by others. However, in a group of four cholesterol-fed chronic animals studied ten days after the ADP infusion, no cellular damage was found at histology.

We conclude that despite these anecdotal observations the cholesterol diet does not enhance in rabbits the effect of platelet emboli due to selective carotid ADP infusion on cerebral circulation.

Conclusions

Selective ADP infusion in the internal carotid artery of rabbits (at a final concentration in blood of $10^{-3}$ M) produces platelet emboli and multiple transient ischemic foci in the intracranial distribution of the internal carotid artery.

The platelet aggregation thus obtained is constant, and emboli of sufficient size to temporarily occlude intracranial arterioles and produce tissue ischemia are formed.

The reproducibility of effects in this experimental model is high, although time pattern, size, and number of ischemic foci show some variability. More long-lasting effects, however, are limited to persistent (not permanent) occlusions in a few small branches in about one-third of the subjects, but well compensated through collateral circulation, so that no permanent anoxic damage or cerebral infarctions are visible in animals studied ten days after the infusion of ADP.
EXPERIMENTAL STUDY WITH INTRACAROTID ADP INFUSION IN RABBITS

This is at variance with the observation of Jørgensen et al.\textsuperscript{12,13} in the kidney and myocardium. Such variant results may be explained by experimental or species difference, or more simply by the presence of a rich collateral circulation in the brain. In our case, variations of the experimental conditions, dose, and duration of ADP infusion do not influence this aspect, as shown in preliminary work.\textsuperscript{5}

It must be stressed that since ischemia may have been favored by defects of collateral circulation due to temporary reduction (actually a drop to 50\%) of mean arterial blood pressure during the initial phase of ADP infusion, it might be advisable to compare the effects of ADP infusion with that of an agent which, when locally infused, provokes a similar platelet aggregation without a large drop in systemic blood pressure, such as arachidonic acid at a very low concentration. Such study is now in progress,\textsuperscript{16} while at present the description of this ADP model seems completed.

In such a model, hypercholesterolemia induced by two months of high cholesterol diet does not modify the pattern of platelet aggregation and of transient emboli and ischemia in the rabbit. In all respects, therefore, the animal model described reproduces the mechanism of human transient ischemic attacks due to platelet emboli.

References

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