Brain creatine kinase in blood after acute brain injury

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SYNOPSIS Severe cold injury of the brain increased significantly both total creatine kinase and the corresponding brain isoenzyme (CKbb) activity in confuens sinuum samples. CKbb could be detected also in peripheral blood a few hours after severe brain injury in eight of 12 patients. Finding of CKbb in human plasma may prove a useful indicator of severe brain injury.

Acute tissue damage usually releases intracellular enzymes into the circulation. Acute brain damage is followed by increased creatine kinase (CK) activity in the serum (Acheson et al., 1965; Langton et al., 1967; Eisen and Sherwin, 1968; Wolintz et al., 1969). Efforts to determine the source of increased CK activity have given surprising results: typical brain tissue isoenzyme, CKbb, has not been found at all in the serum. Rather the increased total CK activity was composed of the isoenzymes of skeletal muscle and heart; CKMM and CKMB (Dubo et al., 1967; Cao et al., 1969). Serum enzyme diagnosis has therefore been considered useless for the diagnosis of brain damage.

New and more sensitive CK isoenzyme methods have been developed (Roe et al., 1972; Somer and Konttinen, 1972) offering new possibilities for organ specific enzyme analysis (Klein et al., 1973; Konttinen and Somer, 1973; Somer et al., 1973). We therefore studied whether these more refined methods would reveal release of CKbb into blood after acute brain injury in both experimental animals and in human cases.

METHODS

EXPERIMENTAL SERIES Healthy rabbits were used. The first blood sample was taken from an auricular vein (BA sample) and the animals were then anaes- thetized with 30–35 mg sodium pentobarbitone (Nembutal) per kg body weight. Atropine was always used. Local anaesthesia for tracheostomy was induced with a 1–2% solution of prilocaine (Citanest). The animals breathed spontaneously throughout the experiment. Procaine penicillin (300 000 iu) was given intraperitoneally to prevent wound infection. Peripheral blood samples were taken through a catheter inserted into a jugular vein with the cephalad end of the vessel ligated. For cerebral blood samples a cannula was attached to the confuens sinuum (Troupp et al., 1966). One peripheral and one cerebral sinus sample were taken immediately after the surgical preparation was completed (AP sample). A funnel with a diameter of 15 mm was attached to the left of the sagittal suture of the rabbit skull, just behind the coronary suture. A severe cold injury was induced in 15 animals by pouring liquid nitrogen into the funnel (Kaste and Troupp, 1972). The freezing time was one and a half minutes. Blood samples were drawn three, six, and 12 hours after the injury. Twelve control animals underwent all procedures except the brain injury.

CLINICAL SERIES Twelve patients with severe brain injury suffered within the previous eight hours, and seven patients undergoing neurosurgical operations were studied (see Table 2). The indications for the neurosurgical procedures were: middle cerebral aneurysm (two patients), deep meningoima (two patients), malignant intracerebral tumour (two patients), and one acoustic neuroma. In all instances there was some trauma to brain tissue during the operation. Serial peripheral blood samples were taken, but a rigid time schedule could not always be achieved with the brain injured patients.

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TABLE 1
PLASMA TOTAL CK AND CKBB ISOENZYME ACTIVITY AFTER EXPERIMENTAL BRAIN INJURY*

<table>
<thead>
<tr>
<th>Cerebral sinus</th>
<th>Peripheral blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total CK (iu/l)</strong></td>
<td><strong>CKBB isoenzyme (iu/l)</strong></td>
</tr>
<tr>
<td><strong>(No.)</strong></td>
<td><strong>(No.)</strong></td>
</tr>
</tbody>
</table>

Before anaesthesia
Expt. | Control
---|---
515 ± 197 | 383 ± 161 |
406 ± 95  | 312 ± 99  |
15 | 15 | 15 | 15

After preparation

3 Hours after injury 12
Expt. | Control
---|---
384 ± 84 | 236 ± 45 |
401 ± 110 | 215 ± 29 |
15 | 15 | 15 | 15

Exp. | Control
---|---
350 ± 55 | 238 ± 58 |
399 ± 139 | 292 ± 38 |
15 | 15 | 15 | 15

TABLE 2
PLASMA CREATINE KINASE ISOENZYMES IN CLINICAL BRAIN INJURY

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Clinical data</th>
<th>Samples (hours after trauma)</th>
<th>CKBB isoenzyme (iu/l)</th>
<th>Total CK* (iu/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cerebral contusion</td>
<td>Intellectual impairment</td>
<td>5</td>
<td>—</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Cerebral contusion</td>
<td>Unconscious for 10 d</td>
<td>3</td>
<td>14</td>
<td>274</td>
</tr>
<tr>
<td>3</td>
<td>Concussion</td>
<td>Conscious when brought in</td>
<td>—</td>
<td>1030</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Cerebral contusion, intra-cerebral haematoma</td>
<td>Unconscious for 2 m</td>
<td>4</td>
<td>3-10</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>Cerebral contusion</td>
<td>Unconscious for 2 m, slight improvement later</td>
<td>5</td>
<td>3-10</td>
<td>230</td>
</tr>
<tr>
<td>6</td>
<td>Gunshot wound of head</td>
<td>Dead 22 h after injury</td>
<td>6</td>
<td>3-10</td>
<td>363</td>
</tr>
<tr>
<td>7</td>
<td>Concussion</td>
<td>Conscious; a few seizures after injury</td>
<td>8</td>
<td>3-10</td>
<td>147</td>
</tr>
<tr>
<td>8</td>
<td>Cerebral contusion</td>
<td>Unconscious for 5 w</td>
<td>2.5</td>
<td>88</td>
<td>445</td>
</tr>
<tr>
<td>9</td>
<td>Cerebral contusion</td>
<td>Dead 10 h after injury</td>
<td>12</td>
<td>1575</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Cerebral contusion</td>
<td>Intellectual impairment</td>
<td>8</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Brain injury</td>
<td>Unconscious for 3 w. Intellectual impairment</td>
<td>6</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Acute subdural haematoma</td>
<td>Dead 15 h after injury</td>
<td>1.5</td>
<td>3-10</td>
<td>76</td>
</tr>
</tbody>
</table>

* Normal values for total CK: 0-50 iu/l.

* Mean ± SE; no. = number of samples.
BIOCHEMICAL METHODS  Blood samples were taken into heparinized tubes. Some haemolysis occurred in most tubes. Glutathione was added to achieve a 10 mmol/l concentration in plasma. The samples were either analysed immediately or stored at −20°C and analysed within two months.

Total CK activity was measured by means of test kits (CPK activated, Boehringer, Mannheim), with a normal range of 0–50 iu/l in human plasma. Creatine kinase isoenzymes were separated electrophoretically and determined by a fluorescence technique (Somer and Konttinen, 1972). The method detects an isoenzyme of 3–5 iu/l. A linear quantitation is achieved within 10–250 iu/l, if a short incubation time (0.5 h) is used. For higher activities corresponding dilutions were made. The results are expressed as CK_{BB} activity (iu/l).

RESULTS

The cold injury caused a rapid rise in CK activity in cerebral sinus blood. Within three hours total CK activity rose by 397 iu/l in the injury group but only by 48 iu/l in the control group. The difference is statistically significant (P<0.01, Student's t test). The rise is mainly due to CK_{BB} activity, which rose by 266 iu/l in the injury group and by only 14 iu/l in the control group (P<0.02). The highest CK_{BB} values were seen in the six hour and 12 hour samples from the confluence sinuum. No rise in CK_{BB} activity was observed in peripheral blood samples collected at the same time (Fig. 1, Table 1).

Peak total CK activity was measured at 12 hours. The animals surviving this long showed a wide scatter in enzyme values and no clear difference was noted between experimental and control animals, either in cerebral sinus or in peripheral blood samples. CK_{MM}, the isoenzyme of skeletal and heart muscle, was usually responsible for high total CK values in the 12 hour samples.

FIG. 2 Plasma creatine kinase isoenzyme in a patient with cerebral contusion. The CK_{BB} isoenzyme is typical of brain tissue, and CK_{MB} and CK_{MM} are typical of heart and skeletal muscle. The first sample (left) was taken 2.5 hours after the injury. Total CK activity was 445 iu/l (normally less than 50 iu/l), and CK_{BB} made up 20% of the total CK activity. The second sample (middle) was taken six hours after the injury. Total CK activity was 740 iu/l, and CK_{BB} was still visible. The third sample (right) was taken 12 hours after the injury. Total CK activity was 1575 iu/l, and CK_{BB} could no longer be demonstrated.

FIG. 1 Plasma creatine kinase_{BB} isoenzyme levels (mean ± SEM) in the rabbit after a severe local freeze injury to the brain.
CLINICAL  In patients with acute brain injury CK$_{BB}$ isoenzyme was found in peripheral blood in eight of 12 patients. All eight patients had had severe brain injury, usually a cerebral contusion, causing severe disturbance of consciousness (Table 2). CK$_{BB}$ activity was usually at its peak in the first sample and then disappeared quickly (Fig. 2). At most it was 23% of total CK activity. The peak total CK activity was found later when there was little or no CK$_{BB}$ activity. No CK$_{BB}$ activity was demonstrated in samples collected after the neurosurgical operations.

DISCUSSION

In the experimental rabbits the rise in total CK activity in cerebral venous blood was largely due to CK$_{BB}$ activity, the isoenzyme which occurs mainly in the brain. Peripheral blood did not show this rise in CK$_{BB}$ activity; perhaps the amount of CK$_{BB}$ released from the damaged brain was too small to cause an appreciable rise in CK$_{BB}$ in pooled peripheral blood. The rise of CK$_{BB}$ in cerebral venous blood alone, and not in pooled peripheral blood, excludes lungs, spleen, kidneys, red muscle or thyroid as sources of this CK$_{BB}$, although in the rabbit these organs contain some CK$_{BB}$ (Brody and Hatcher, 1967; Sherwin et al., 1967). Total CK activity increased in quite a few samples of both cerebral and pooled peripheral blood 12 hours after injury, but this was due to a rise in CK$_{MM}$ or CK$_{MB}$, the isoenzymes presumably released by surgical trauma, anaesthesia, or impaired ventilation (Dixon et al., 1971; Phornphutkul et al., 1974).

The clinical results tally with the experimental ones. Some CK$_{BB}$ is released from brain to blood soon after a severe brain injury. Since normal human plasma does not show any CK$_{BB}$ activity, small amounts can easily be detected in analyses of peripheral blood. To find CK$_{BB}$ in peripheral blood after clinical brain injury requires a sensitive method as well as well-timed sampling. The fluorescence technique (Somer and Konttinen, 1972) is clearly more sensitive than the methods used in previous studies (Dubo et al., 1967; Cao et al., 1969). When present, CK$_{BB}$ isoenzyme could usually be detected even in the earliest samples collected. No association between the presence of CK$_{BB}$ and total CK activity could be observed. This again shows the uselessness of total CK measurements as an indicator of brain damage.

The clinical value of plasma CK$_{BB}$ determinations has so far not been determined. Although CK$_{BB}$ occurs in some other human organs too (Dawson and Fine, 1967), it has not been found previously in human plasma in any other clinical conditions except in a few cases of malignant hyperpyrexia (Zsigmond and Starkweather, 1973). It seems that a severe injury is needed to cause the release of CK$_{BB}$ into peripheral blood and so, when it does, this may possibly offer new criteria for the early assessment of the severity of brain damage.

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