Cerebrospinal fluid enzymes in acute brain injury

3 Effect of hypotension on increase of CSF enzyme activity after cold injury in cats

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SUMMARY The influence of trimetaphan-induced hypotension was studied on the increase in the activities of various enzymes in the cerebrospinal fluid after cold injuries of the brain in cats. Hypotension was induced immediately after freezing, and in a second series after a delay of 45 minutes. It was shown that induction of hypotension may inhibit the appearance of enzymes in the CSF after cold injuries in the first seven hours after freezing. Histological studies revealed less pronounced oedema in the hypotensive animals. The results suggest that hypotension retards the transport of enzymes released from necrotic areas through the extracellular fluid towards the CSF.

Previous work has demonstrated increased activities of the enzymes creatine phosphokinase (CPK), α -hydroxybutyric acid dehydrogenase (α -HBDH), lactate dehydrogenase (LDH), aspartate aminotransferase (GOT), and pseudocholinesterase (ChE) in the cerebrospinal fluid (CSF) after cold lesions of the brain in cats, the increase being related to the severity of the injury (Maas, 1977a,b). The practical value of CSF enzyme determinations as an indication of the extent of brain damage will be highly dependent on the consistency of enzyme transport from the extracellular fluid (ECF) to the CSF. Should transport be hampered in any way, falsely low CSF enzyme activity may result even in the presence of severe brain damage. It seemed not unlikely that brain oedema could promote transport, and we decided to investigate this.

Material and methods

The experimental setting has been described in detail by Maas (1977a). In summary: in healthy, adult, anaesthetised cats weighing 2800–6400 g, otherwise unselected for age or sex, a cold lesion of the brain was induced according to the method of Beks and coworkers (1965).

Before and after freezing, CSF samples of 0.35 ml were withdrawn from a cisternal catheter every half hour. Samples were analysed for

CPK, α -HBDH, LDH, GOT, and ChE. Enzyme estimations were performed with the optimized Merck-1-test kits (E. Merck, Darmstadt, Germany).

Arterial blood pressure, end expiratory CO_2 , ECG, ventricular and cisternal fluid pressure were monitored continuously and measured every half hour. Respiratory frequency and heart rate were calculated.

The first three samples of CSF were discarded to eliminate false high enzyme levels due to contamination during placement of the cisternal catheter. Control values were established for each experiment by averaging the values obtained in the period one to two and a half hours after beginning cisternal taps—that is, CSF samples 4–6. Thereafter a cold lesion of the brain was induced in 29 of the 36 experiments. Seven animals served as a control group. In 15 animals artificial hypotension was induced using trimetaphan (Arfonad) in a concentration of 0.5–1.0 mg/ml. The mean arterial blood pressure was reduced to 75–90 mmHg.

The experiments were divided into four series: *Series 1*: control series (seven animals). These animals received a complete sham operation, without cold lesion or hypotension.

Series 2: in 14 animals cold injuries were induced by freezing 1.34 cm² of cortex in the left frontoparietal region for six minutes exactly at -40°C . No hypotension was effected.

Series 3: in this series (15 animals) cold lesions

were induced as in series 2, and then hypotension was induced.

3a: in series 3a (six animals) the blood pressure was lowered immediately after freezing.

3b: in series 3b (nine animals) lowering of the blood pressure was delayed until 45 min after induction of the cold lesion.

The experiments were ended after an arbitrarily chosen time limit of 7.25 hours after induction of the cold lesion, or earlier if brain stem herniation occurred. One animal in each series was studied longer.

After the experiments the animals were killed and the brains removed and fixed in a 4% formalin solution for two weeks. Paraffin sections were cut through the maximal diameter of the lesion and stained with haematoxylin-eosin (HE), periodic acid Schiff (PAS), trichrome PAS, and according to the method of Klüwer-Barrera.

STATISTICAL ANALYSIS

The differences between the values at the different time levels—that is, in the period 0.25 to 7.25 hours after induction of the lesion—and the control values for the individual experiment were calculated. Statistical analysis was performed on these at every time level—that is, every half hour—using Wilcoxon's ranking test. The following series were compared: (A) control versus cold injury—control versus series 2, control versus series 3a, and control versus series 3b; (B) cold injury versus cold injury and hypotension—series 2 versus series 3a, and series 2 versus series 3b; and (C) immediate versus delayed hypotension—series 3a versus series 3b.

Results

The systemic effects of trimetaphan and the effect of hypotension on the development of increased ventricular fluid pressure after cold injuries in cats have been described elsewhere (Maas and de Lange, 1977). The results reported in this paper concentrate on the effect of hypotension on the changes in CSF enzyme activity.

CSF ENZYMES AFTER COLD INJURY—NO HYPOTENSION (SERIES 2)

Five of the 14 animals in this series developed brain-stem herniation within two and a half hours of induction of the cold lesion. The survival time in these animals was too short for changes in CSF enzyme activity to occur. Hence they were excluded from further analysis.

A significant increase (Wilcoxon $P < 0.01$) of the activity of all enzymes studied in the CSF was

demonstrated in the remaining nine animals. All enzymes showed a similar pattern of response. The dynamics of these changes have been described previously (Maas, 1977a). The results obtained for CPK are illustrated in Fig. 1.

Fig. 1 Scatter diagram showing levels of CPK activity observed in the CSF of cats after induction of a cold lesion to the brain. These animals were not treated with hypotension.

All animals which survived the cold injury for more than two and a half hours, except one, developed increased CSF enzyme activity.

CSF ENZYMES AFTER COLD INJURY AND IMMEDIATE HYPOTENSION (SERIES 3a)

No appreciable changes in the activity of the enzymes studied in the CSF were noted in three of the six animals in this series. Three animals developed a slight increase in CSF enzyme activity, but this rise occurred later and was far less pronounced than in the series without hypotension. The results for CPK are illustrated in Fig. 2. The other enzymes exhibited a similar pattern.

CSF enzyme values in series 3a did not differ significantly from the control series. The levels of all CSF enzymes studied in series 3a were significantly less elevated (Wilcoxon $P < 0.01$) than in series 2.

CSF ENZYMES AFTER COLD INJURY AND DELAYED HYPOTENSION (SERIES 3b)

In series 3b induction of hypotension was delayed until 45 min after induction of the cold lesion. Within these first 45 min the ventricular fluid pressure (VFP) had already risen to values

Fig. 2 Scatter diagram showing levels of CPK activity observed in the CSF after induction of a cold lesion to the brain in cats in which hypotension was effected immediately after freezing. The effect of induction of hypotension on development of raised CPK levels in the CSF after freezing becomes evident when Figs. 1 and 2 are compared.

of 7.6–17.5 mmHg (control values 0.8–5.8 mmHg). One animal developed a progressive gradient between the ventricular and cisternal fluid pressure shortly after induction of hypotension and was excluded from further analysis as an early interruption of flow from ventricle to cistern seemed probable.

Of the remaining eight animals, five did not develop any appreciable changes in CSF enzyme activity at all. One experiment was terminated prematurely because of mechanical difficulties with the cisternal catheter. Two animals did develop a considerable increase in activity of all CSF enzymes. One of these was studied for an additional six hours under hypotension. The pattern of changes in the activities of the enzymes CPK and α -HBDH in the CSF are shown in Fig. 3.

The rapid increase and fall of CSF enzyme activity observed in this animal suggests that the greatest changes occur within 12 hours after injury.

The overall CPK results for series 3b are illustrated in Fig. 4.

The enzymes CPK, α -HBDH, LDH, and GOT in the CST were significantly raised (Wilcoxon $P < 0.05$) above the control series. ChE was not. Statistical analysis of the enzyme differences between series 2 and 3b revealed at most a tendency for the lower enzyme levels to occur in the animals with hypotension. This lack of significance was due mainly to the presence of the non-responding

Fig. 3 Changes in the activities of the enzymes CPK and α -HBDH in the CSF of animal 59. This animal demonstrates a considerable rise in the activities of both enzymes, although hypotension was effected three-quarters of an hour after induction of a cold lesion to the brain.

Fig. 4 Scatter diagram showing levels of CPK activity observed in the CSF after induction of a cold lesion to the brain in cats in which hypotension was effected three-quarters of an hour after freezing. The effect of delayed hypotension on development of raised CPK levels in the CSF after induction of cold lesions to the brain is evident when Figs. 1 and 4 are compared.

animal in series 2. When this animal was excluded from further analysis, the difference became significant for all enzymes (Wilcoxon $P < 0.05$). No statistical difference existed between the series with delayed and the series with immediate hypotension.

HISTOLOGICAL STUDIES

The histological changes in the brain after induction of a cold lesion have been well-documented (Klatzo *et al.*, 1958; Clasen *et al.*, 1962; Go *et al.*, 1967). The lesion induced in our studies resulted in a relatively large zone of focal necrosis and development of extensive cerebral oedema, confined to the ipsilateral hemisphere. Oedema was evident by less intense staining and dispersion of the myelin fibres.

The induction of hypotension resulted in two marked differences: less oedema, and fewer haemorrhages in the necrotic zone. Only four of the 15 animals in the series with hypotension (series 3a and 3b) exhibited extensive brain oedema. These four animals had developed increased CSF enzyme levels.

Discussion

It was thought that the amount of enzymes released from injured brain tissue into the CSF would be related to the extent of brain damage, and so could be of prognostic value (Smith *et al.*, 1960; del Villar *et al.*, 1973). However, the prognostic value of CSF enzyme levels must depend also on the consistency with which enzymes released from damaged or necrotic brain cells are transported to the CSF.

Previous studies on changes in CSF enzyme activity after a cold lesion to the brain in cats demonstrated a considerable rise of the enzymes CPK, α -HBDH, LDH, GOT and ChE in the CSF of eight out of nine animals surviving more than two and a half hours after induction of the lesion (Maas, 1977a). One animal did not respond to the injury with any rise of CSF enzyme activity at all. The only factor that distinguished this animal was a lower blood pressure. It was considered possible that this might be the cause of the lack of CSF enzyme response. The same phenomenon was also noted in another series of experiments (Maas and Beks, 1974, unpublished). Further research into the relation between arterial blood pressure and CSF enzyme levels after brain injuries seemed indicated.

The results reported here suggest that lowering the arterial blood pressure delays or even inhibits the appearance of enzymes in the CSF in the acute phase after brain injury (Table).

Although no changes in CSF enzyme activities were observed after the cold lesion in more than half of the animals with hypotension, the expected cold lesion was present and necrosis was marked. It is inconceivable that no enzymes were then released into the ECF. However, these enzymes

Table Number of animals developing changes in CSF enzyme activity after a cold injury in the series with and without hypotension

Series	No change	Evident increase
No hypotension (series 2)	1	8
Immediate hypotension (series 3a)	3	2
Delayed hypotension (series 3b)	5	2

did not appear in the CSF, and it seems likely that the transport of enzymes towards the CSF was delayed or inhibited.

Transport through the ECF towards the CSF is in theory possible by way of simple diffusion, by bulk flow of ECF, by flow of oedema fluid, or by a combination of these. Klatzo *et al.* (1967) have demonstrated that the spreading of oedema can be slowed by inducing hypotension. Our studies also revealed less oedema in the animals with hypotension. In four hypotensive animals oedema was more obvious. In these animals the activity of CSF enzymes was considerably raised.

Enzymes are probably transported more slowly through the ECF towards the CSF in the absence of brain oedema. Apparently the process of diffusion, or of normal bulk flow of ECF, is not sufficient to transport the enzymes from the necrotic site of the brain towards the CSF within the first seven hours after injury.

Cserr and Ostrach (1974) have demonstrated a bulk flow of ECF towards the CSF in the presence of brain oedema. Our results seem to confirm this, but would also indicate that bulk flow of ECF towards the CSF is either slow or absent when brain oedema is slight.

Conclusions

Studies on the changes in CSF enzyme activity after cold lesions of the brain in cats made hypotensive indicate that no increase of the enzyme levels occurs in the CSF in the first seven hours after injury, in the absence of extensive brain oedema, even although extensive cortical necrosis is present. This means that early determination of CSF enzymes in patients with severe brain injuries will only be of prognostic value when there is a combination of brain damage and extensive brain oedema.

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