Enzyme response of traumatized tissue after intracortical injection into 5 day old rat brain

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SUMMARY Penetration of a microneedle and injection of 4 μl saline into the neocortex of the 5 day old rat brain produced no changes in behaviour of the rats up to 21 days post-injection. Within 24 hours sections indicated that tissue damage was apparent only at the pia-arachnoid membrane and where fluid was released; elsewhere the needle pathway was identified by the enzyme response. The enzyme histochemistry showed a marked increase in glial cell activity of some phosphatases within 24 hours at the site of injury; the pia-arachnoid and outer limiting membrane also showed abnormally high phosphatase reactions. NADH₂-diaphorase was the only dehydrogenase that was raised in some nerve and glial cells at 24 hours post-injection but other dehydrogenases, mainly LDH and SDH, showed changes at four days post-injection. The phosphatases and 5'-nucleotidase previously showing intense glial cell enzyme reactions appeared to reach peaks of activity at eight days, and at 16 days the onset of scarring was apparent. In the pia-arachnoid enzyme activity increased to 21 days. Some enzymes, particularly AChE and MAO, showed no alterations of note throughout.

Work is being reported on behavioural, morphological, and metabolic changes and biosynthetic mechanisms in the developing and mature central nervous system (CNS) after electrical stimulation or administration of substances intracerebrally. In such experiments penetration of a microneedle or an electrode into the cerebrum will produce a compression front and displacement of cells with consequent disturbances to their metabolism. Small volumes of fluid released into the tissue will produce changes in ionic strength, electrolyte concentration, pH, temperature, and breaking of colloidal suspensions, while inflammatory and other less clearly defined processes resulting from trauma are also likely to occur.

An earlier communication (Robinson, 1969) reported the disturbances to adult brain cell enzyme activity produced by intracerebral penetration of a microneedle. Responses of enzymes to injury and their changes in activity during recovery within the different cells and intracellular structures of the neocortex in the rapidly developing 5 day old rat brain will be expected to show substantial differences from the adult. The enzymes controlling important pathways of cerebral metabolism in the region of traumatized tissue of the 5 day old rat neocortex after microneedle penetration and injection of 4 μl saline were examined at intervals from one to 21 days post-injection.

METHODS Male Sprague-Dawley rats, 5 days old and weighing 7.5–8 g were lightly anaesthetized and hair shaved off the scalp. A sagittal skin incision was made and the skull scraped clean under aseptic conditions. The animal was placed in a stereotaxic instrument and a small piece of skull, which was very thin, immediately above the origin of the corticospinal tract was removed on one side of the sagittal suture. Using a Hamilton syringe, coupled to a fine glass needle, approximately 150 μ diameter, 5 μl 0.325M sodium chloride (isotonic with respect to blood serum) were injected into the cortex over a period of 15 minutes by a motor-driven micrometer. The wound was sutured, covered with Nobecutane (Duncan Flockhart and Evans, London), and the animals allowed to survive for 1, 2, 3, 5, 8, 16, and 21 days post-injection.

The brains were removed, rapidly frozen and fresh
frozen sections 10 μ thick, cut at −17° on a cryostat. The techniques for demonstrating enzyme activities have been described (Robinson, 1969); the enzymes were NADH₂-diaphorase, lactate (LDH), succinate (SDH), glucose 6-phosphate (G6-PDH) and α-glycerophosphate (αGPDH) dehydrogenases, acid phosphatase, Mg²⁺-adenosine triphosphatase (Mg-ATPase), 5'-nucleotidase, thiamine pyrophosphatase, acetylcholinesterase (AChE), and monoamine oxidase (MAO).

RESULTS

All the animals recovered from the operation and showed no signs of abnormal behaviour before they were killed.

The furrow ploughed by the needle was identified up to eight days post-injection by clusters of cells, invariably forming a broken line, exhibiting an increase in enzyme activity. Beyond eight days, identification of tissue damage by this method was more difficult due to overgrowth in the rapidly developing brain. A fringe of debris, surrounding an area of tissue necrosis where saline was released, persisted up to 16 days but seldom to 21 days post-injection.

TWENTY-FOUR HOURS POST-INJECTION The enzymes acid phosphatase (Figs 1a, b, c) and Mg-ATPase exhibited a moderate reaction, not normally present at this age, in nerve cells, microglia, and enlarged astrocytes in a small well-defined but fragmented band along the line of penetration; the neuropil acid phosphatase was also slightly raised adjacent to damaged tissue. There appeared to be an increase of 5'-nucleotidase in large cells approximating very closely to tissue destruction but rapidly diminishing away from this region. NADH₂-diaphorase was the only dehydrogenase responding significantly to injury at one day; the enzyme was raised in the cytoplasm of some nerve cells and a few glial cells and lower in neuropil adjacent to injury (Fig. 2). Other dehydrogenases showed no change except within a few isolated glial cells which were more reactive than normal. MAO exhibited a loss of activity in neuropil where it is normally moderate in intensity at this time (Fig. 3). AChE showed no response.

At the surface the pia-arachnoid and outer limiting membrane deep to pia mater showed
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FOUR DAYS POST-INJECTION Some dehydrogenases showed a response not previously apparent. SDH and LDH were raised in glial cells scattered around necrosis where fluid was injected but in the path of needle penetration the dehydrogenase response in these and other cells appeared normal. The glial cells exhibiting strong acid phosphatase and Mg-ATPase activity were less numerous than at earlier times in the region of necrosis where saline was released (Fig. 6); intermittent clusters of glial cell Mg-ATPase activity were similar to those seen at 24 hours post-injection.

FORTY-EIGHT HOURS POST-INJECTION Acid phosphatase and Mg-ATPase had increased to an intense reaction within a greater number of reactive glial cells. Mg-ATPase was more intense in blood vessels in the injured zone. Thiamine pyrophosphatase, normally confined to a weak to moderate well-defined activity in nerve cells and blood vessels at 5 days old, exhibited a strong reaction in scattered microglia around tissue necrosis at this time; nerve cells close to this region appeared to be normal (Fig. 4). The 5'-nucleotidase reaction was prominent in hyperactive swollen glial cells and in smaller round cells (Fig. 5). The response of NADH$_2$-diaphorase was the same as at 24 hours, while other dehydrogenases and AChE did not respond. Accumulations of raised poorly localized MAO activity seen as fragmented thin lines of dark staining were thought to result from cell breakdown and diffusion from the neuropil.

Reactions in the pia-arachnoid membrane were similar to those seen at 24 hours post-injection.

FIG. 2. One day post-injection. NADH$_2$-diaphorase. Loss of activity in neuropil and nerve cell bodies adjacent to injury at left hand side of figure but slightly raised in a few glial cells and the cytoplasm of nerve cells (down centre). ×910.

FIG. 3. One day post-injection. MAO. Depletion of nerve cell and neuropil activity in limited area at site of release of saline (strong border reaction). ×910.
activity were still apparent in the path of needle penetration. The 5’-nucleotidase reaction was more intense in large, more clearly defined glial cells seen against a background of neuropil activity which was paler than at two days; nerve cells and fibres continued to show a normal weak reaction.

The pia-arachnoid and glial cells deep to the limiting membrane exhibited a raised Mg-ATPase reaction in addition to acid phosphatase, thiamine pyrophosphatase, and 5’-nucleotidase observed at 24 hr; there was no apparent change in dehydrogenase activity.

**EIGHT DAYS POST-INJECTION** NADH$_2$-diaphorase and SDH were still raised in a few scattered glial cells but the neuropil reaction, low at 24 hours adjacent to injury, was restored to normal intensity at eight days. The intense acid phosphatase, thiamine pyrophosphatase, and Mg-ATPase activities previously seen in glial cells appeared less prominent and diffuse within the cytoplasm, suggesting that these enzymes were past their peak of activity in these cells (Fig. 7).

The 5’-nucleotidase reaction was as intense as before within the microglia and astrocytes. The site of released saline was still identified by severe necrosis but the appearance of scarring was becoming evident here and in the path of needle penetration which was difficult to locate due to sparsity of glial cells with abnormally high enzyme activities.

All enzymes catalysing the release of phosphate gave prominent reactions in the pia-arachnoid membrane at the site of needle penetration; a few glial cells in the immediate subjacent cortex

**FIG. 4.** Two days post-injection. Thiamine pyrophosphatase. Increase in microglial cell and neuropil activity at site of release of saline seen as latticework of damaged tissue. Normal reaction in nerve cells down left hand side. × 360.

**FIG. 5.** Two days post-injection. 5’-Nucleotidase. Strong abnormal reaction in nerve cells (arrows) and surrounding glial cells adjacent to site of injection at right. × 910.
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FIG. 6. Four days post-injection. Mg$^{2+}$-ATPase. Some enlarged astrocytes exhibiting an increased cytoplasmic activity (arrows), other structures normal. Complete necrosis at site of release of saline (marked X). x 360.

FIG. 7. Eight days. Mg$^{2+}$-ATPase. Path of needle penetration (down centre) still identifiable by few hyperactive glial cells. x 145.

FIG. 8a. 21 days. LDH. Pallor of neuropil activity but survival of nerve cell cytoplasmic activity seen within the pale region below damaged pia-arachnoid membrane. x 245. FIG. 8b. Higher magnification of FIG. 8a showing nerve cell cytoplasmic activity (arrows) surrounded by weak neuropil activity increasing to normal in bottom right and left corners. x 910.
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FIG. 9. 21 days. NADH$_{2}$-diaphorase. Fewer surviving nerve cells (arrowed) but better neuropil and glial cell response than for LDH. $\times 620$.

SIXTEEN AND 21 DAYS POST-INJECTION  Scarring was seen in regions of injury which previously demonstrated abnormally high enzyme activity. No abnormal glial cell dehydrogenase reactions previously observed were apparent. Many fewer swollen hyperactive glial cells showing abnormally strong acid phosphatase, thiamine pyrophosphatase, and Mg-ATPase activities were seen in the line of needle penetration but cells retaining the abnormally high 5'-nucleotidase reactions were numerous. Reactions of all the enzymes in nerve cells adjacent to injury but showing morphological integrity appeared normal.

The pia-arachnoid membrane exhibited increases in dehydrogenase activities. In this region LDH showed a pallor in a semicircle of subjacent neuropil and glia but nerve cell cytoplasm exhibited a moderate reaction similar to normal cells (Figs 8a and b); NADH$_{2}$-diaphorase (Fig. 9), SDH, GPDH, and G6-PDH did not show the pallor in neuropil and glia. Acid phosphatase (Fig. 10), thiamine pyrophosphatase (Fig. 11), and 5'-nucleotidase were very
strong in the pia-arachnoid membrane and in a crescent-shaped subjacent region of varying sizes of glial cells. The reaction for Mg-ATPase was less intense but still abnormally high in the pia-arachnoid membrane but subjacent injured neocortex showed a marked diminution of cells hyperactive in Mg-ATPase (Fig. 12).

**DISCUSSION**

Tissue injury resulting from intracerebral injection into the motor cortex of the 5 day old rat could be identified histologically only by a localized area of damage to the pia-arachnoid membrane at the point of entry and also by tissue necrosis where 4 µl saline had been released. At the site of entry indentation or fragmentation of pia-arachnoid membrane was apparent for a short distance, less than twice the width of the needle, either side of the point of entry. At the site of release of saline an oval area of tissue necrosis was present. Between these two regions, where the needle had ploughed through the cortex, tissue damage was not clearly identified but the indirect evidence of the intracellular enzyme response to injury was clearly in evidence. The changes in enzyme activity, mainly within glial cells but also within some nerve cells, reflected the metabolic alterations within these cells after disturbance.

Significant changes in enzymes catalysing the release of phosphates were apparent within 24 hours of injection. These enzymes increased substantially above the normal level of activity and were localized mainly within microglia and swollen astrocytes. Nerve cell enzyme response was less marked and confined to fewer cells; it was usually seen as either a loss of localization or a depletion in activity, seldom were enzyme activities increased. The number of cells showing hyperactivity increased during the first four days, the enzyme activity within these cells also increasing with time although at different rates. The differentiation of reactive glia and neuropil became more distinct with increasing time as the enzyme activity in neuropil, above the normal at 24 hours, diminished to a normal level several days later.

It was of particular interest to observe that there were no immediate increases in dehydrogenase activities within nerve cells, glial cells, or...
neuropil in response to injury to the 5 day old rat cortex. This is in strong contrast to the marked increases in these enzymes within the adult rat brain where these changes indicated that above normal demands were being made on oxidative metabolism (Robinson, 1969). The sensitivity of some dehydrogenases to injury within the neuropil was apparent by a pallor in the damaged regions where other enzymes localized within glial cells were hyperactive. The pattern of enzyme changes remained similar up to eight days but the population of hyperactive cells markedly diminished with evidence of scarring. However a reversal of this trend was seen in the pia-arachnoid and outer limiting membrane deep to pia mater where enzyme activity increased considerably above the normal at the site of needle penetration; in this region the dehydrogenases also showed a greater response than in the neocortex. Enzyme activity continued at the same abnormally high level up to 21 days post-injection in the pia-arachnoid membrane in contrast with the marked decrease in number of cells exhibiting enzyme hyperactivity in the neocortex. This finding is interesting in view of the fact that both regions are ectodermal in origin.

The reactions of acetylcholinesterase and monoamine oxidase showed no alterations of note in these experiments.

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REFERENCE

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