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Stereotaxic injection of kainic acid into the striatum of rats induces synthesis of mRNA for heat shock protein 70

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Stereotaxic injection of the excitotoxin kainic acid into the striatum of rats has been shown to induce the production of messenger RNA to the 70 kDa heat shock protein. This was evident 2 h after injection and was maximal 24 h after injection of kainic acid. No change in the level of messenger RNA to β-actin was dectected.

Kainic acid; Heat shock protein; Messenger RNA; Neurodegenerative disease; (Striatum)

1. INTRODUCTION

The excitotoxin kainic acid (a rigid analogue of the putative amino acid neurotransmitter, glutamic acid) has been used as a selective lesioning agent to model the aetiology of a number of neurodegenerative disorders [1,2]. The mechanism of its action is not known, but its toxicity is generally thought to be a result of neuronal hyperactivity resulting in cellular hypoxia [3,4]. Some biochemical changes [1,2] which occur following kainic acid lesioning are known, but alterations in cellular proteins which could effect cell viability have not been studied. In this study we report that the stereotaxic injection of kainic acid causes the synthesis of mRNA encoding for the 70 kDa heat shock protein in the damaged striatum.

Heat shock proteins (hsps) are a highly conserved group of proteins [5] whose amounts are increased, concomitant with a decrease in the synthesis of other cellular proteins when cells are exposed to a variety of stresses. These include exposure to elevated temperatures [6], ischaemia [7], adenovirus infection [8] and metabolic inhibitors such as arsenite and amino acid analogues [9]. The increase in the level of expression of the hsps has been suggested to be the result of increased amounts of abnormal intracellular proteins (induced by stress) which induce the production of a regulatory protein which in turn stimulates the synthesis of hsps [10]. The precise function of hsp 70, an hsp frequently studied in stress responses, is unknown, although it has been implicated in nuclear repair processes [11] and protection of RNA splicing from heat-induced disruption [12]. It has also been shown to act as an uncoating ATPase in clathrin-coated vesicles [13].

Degenerating neurones in Alzheimer's, Parkinson's, Pick's and Motor neurone diseases contain inclusions which are labelled by antibodies to ubiquitin, a 8 kDa hsp [14–17] which implies that degenerating neurones exhibit a stress response. It is therefore, of interest to study the mechanism of experimental induction of a neuronal stress response since the changes may illuminate those that occur in neurodegenerative diseases.

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2. MATERIALS AND METHODS

2.1. *Stereotaxic injections*

Stereotaxic injections were performed on male Wistar rats (250 g) using a Kopf manifold with the incisor bar raised 5 mm.

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Kainic acid (1 μl, 1 mg/ml in saline) was injected into the striatum of the right hemisphere over a 3 min period (coordinates L 3.0, V 5.5, AP 1.8 [18]). The same animals received 1 μl of 150 mM saline into the opposite hemisphere which served as control tissue. Further animals received only a unilateral injection of saline. Animals were killed after 30 min, 2, 5, 12, 24 and 50 h and the striatum was carefully removed and immediately frozen in liquid nitrogen.

2.2. *Preparation of RNA for Northern blot analysis*

Total RNA from the striatum was isolated using the guanidine isothiocyanate method [19] and poly(A)$^+$ RNA separated by oligo(dT) column chromatography [20]. The RNA was quantitated by spectrophotometry. For RNA gels, the RNA was denatured in the presence of 2.2 M formaldehyde and 50% (w/v) deionised formamide at 65°C and then separated by electrophoresis on a 1.2% (w/v) agarose gel containing 2.2 M formaldehyde [21]. The RNA was then transferred to nitrocellulose membranes by Northern blotting. The nitrocellulose filters were UV-irradiated for 2 min and prehybridised in a cocktail consisting of 5× SSC (sodium citrate buffer), 50 mM sodium phosphate buffer (pH 6.5), 5× Denhart's solution, 0.1% (w/v) SDS, 50% (w/v) formamide and 250 μg/ml salmon sperm DNA at 42°C [20,21]. Hybridisation was performed in the same buffer at 42°C containing an oligolabelled [22] hsp-70 probe (plasmid pH 2.3 [5], which was a generous gift from Dr R.I. Morimoto, contains a 2.3 kb *Bam*HI/*Hin*dIII fragment which contains the entire human hsp-70 coding region) and an actin oligolabelled [22] probe (actin probe was a gift from Dr D.W. Cleveland and is a plasmid containing a 2.0 kb fragment coding for chick β-actin [23]).

3. RESULTS

Animals which had received a kainic acid injection into one striatum (and saline into the opposite striatum) showed vigorous ipsi- and contraversive circling, and intermittent stereotyped behaviour. Animals which only received saline into one striatum did not show abnormal behaviour on

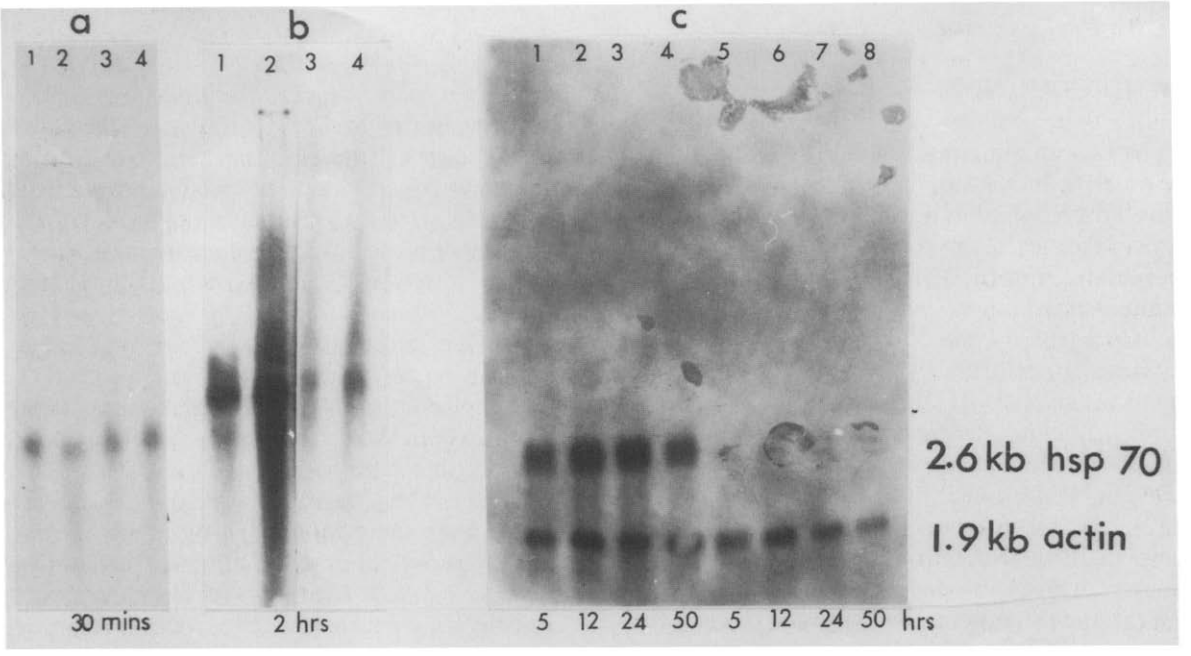

Fig.1. Northern blot analysis. (a) Lane 1 shows the Northern blot of total RNA extracted from a striatum removed 30 min after the injection of kainic acid and lane 3 shows a blot of the opposite striatum of the same animal removed 30 min after the injection of saline. Lanes 2 and 4 represent a duplicate experiment and are blots of striatum removed 30 min after the injection of kainic acid and saline, respectively, from a different animal. (b) Lane 1 shows the Northern blot of total RNA extracted from a striatum removed 2 h after the injection of kainic acid and lane 3 shows a blot of the opposite striatum of the same animal removed 2 h after the injection of saline. Lanes 2 and 4 again represent a duplicate experiment and are blots of striatum removed 2 h after the injection of kainic acid and saline, respectively. After hybridisation of the filters with a ^{32}P-oligolabelled probe to hsp 70, the results show that there is no detectable increase in message to hsp 70 after 30 min, while there is an increase 2 h after injection of kainic acid. (c) Poly(A)$^+$ RNA was isolated from kainic acid (lanes 1–4) and saline (lanes 5–8) injected striatum of rats for Northern blot analysis. mRNA was isolated 5, 12, 24 and 50 h after the injection of kainic acid or saline. The Hybond filter was hybridised to a ^{32}P-labelled hps-70 probe which gives a signal at 2.6 kb and then stripped and rehybridised to an actin probe which gives a signal at 1.9 kb. The photograph for c was obtained by superimposing the autoradiographs of the hsp 70 and actin probed Northerns.

recovery from the anaesthetic. Initially, Northern blot analysis of total RNA isolated from kainic acid and saline injected striatum of the same animals was carried out. These preliminary results showed that 30 min after the injection of kainic acid no increase in the levels of mRNA to hsp 70 occurred (fig.1a), however, 2 h after kainic acid injection an increase in the levels of hsp 70 mRNA was seen (fig.1b), while no change in the levels of actin mRNA was seen in the kainic acid-treated animals (not shown).

To investigate the above results further and to follow later phases of the hsp response, animals were injected with kainic acid and the striatum removed after 5, 12, 24 and 50 h, and mRNA extracted and Northern blot analysis carried out. Fig.1c shows that the hsp response is detectable after 5 h and is maximal at 12 and 24 h, at 50 h there was some apparent decrease in the amount of hsp 70 message detected. With the β-actin probe no significant changes in transcription of actin were seen in the kainic acid-treated animals (fig.1c). These experiments have been performed on three groups of rats and levels of hsp 70 mRNA have been elevated (although to differing degrees) in each experiment. In some experiments the signal corresponding to actin mRNA decreased along with the drop in the level of hsp 70 mRNA in the animals which had received kainic acid for 50 h. This may be due to a loss in total RNA due to extensive cell death occurring over this period.

4. DISCUSSION

The present results show that injection of kainic acid into rat striatum causes a marked increase in the production of hsp 70 mRNA. The mechanism by which stimulation of glutamate receptors causes an increase in hsp 70 has yet to be investigated. Heat shock proteins are thought to be induced by the production of abnormal intracellular proteins which in some way induce a protein factor (already present in the cell) to bind to the hsp promoter. There are several sequelae which may be relevant to the mechanism by which kainic acid induces a heat shock response. Neuronal depolarisation by kainic acid results in a rapid increase in free cytoplasmic calcium due to activation of voltage-independent channels [24]. Increases in intracellular calcium concentrations have been shown to cause cellular damage by several different mechanisms, including activation of intracellular proteases [25,26] and collapse of the mitochondrial membrane potential [27]. Kainic acid also depletes the cellular ATP pool [4], i.e. neuronal death would occur due to local anoxia.

Other mechanisms by which a heat shock response may be generated include free radical attack, and it has been suggested that increased intracellular calcium following kainic acid infusion of cerebellar neurones in culture may activate a serine protease which converts xanthine dehydrogenase to a xanthine oxidase [28]. This latter enzyme then converts inosine and hypoxanthine (whose concentrations are greatly elevated due to ATP catabolism) to uric acid during which free radicals are generated. Evidence for this is that cerebellar neurones in culture are protected from kainic acid toxicity by the addition of superoxide dismutase or mannitol (free radical scavengers) or allupurinol (an inhibitor of the enzyme xanthine oxidase) to the external tissue culture medium [28].

The development of a heat shock response has been shown to be related to intracellular calcium concentrations [30,31], the rapid rise in intracellular calcium due to kainic acid may therefore be the means by which a heat shock response is induced. Alternatively, cellular damage due to proteolysis of free radical attack and/or increased calcium levels may be responsible for the development of an heat shock response.

The question also arises as to whether the heat shock response is produced by neurones or glia. Kainic acid injected in vivo has been shown to result in death of neuronal cell bodies with retention of axons in the affected area; furthermore glia and fibroblasts have been shown to survive kainic acid injected in vivo [2–4,26]. The likelihood is, therefore, that the observed heat shock response is localised to neurones. This can be tested by an in situ hybridisation study of kainate-lesioned brain.

When injected in vivo, excitotoxins such as kainic acid can induce lesions with some of the pathological features of Huntington's, Alzheimer's and Parkinson's diseases [1,2,32]. Several other naturally occurring and man-made toxins are implicated in neurodegenerative diseases. These include Lathyrism, a form of epidemic spastic paraplegia induced by consumption of β-*N*-oxalylamine-L-alanine (BOAA), present in chickling pea

seeds [33] and Guamanian ALS-PD dementia which may be related to consumption of β-*N*-methylamino-L-alanine (BMAA) present in cycads [34]. The pethidine analogue, methylphenyltetrahydropyridine (MPTP), induces a Parkinsonian syndrome in man and primates [35]. Although it is not an excitotoxin, its mechanism of toxicity may involve the generation of free radicals [36]. In addition, ubiquitin, another member of the family of heat shock proteins, labels intracellular protein inclusions in Parkinson's disease, Alzheimer's disease and motor neurone disease [14–17], implying that a heat shock response may occur in the affected neurones in these diseases. Therefore, further investigation of the mechanism by which kainic acid induces neuronal cell death and a heat shock response is important since it may provide clues about the biochemical changes that contribute to cell death in these human conditions.

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