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Arachidonic Acid Inhibits Uptake of Glutamate and Glutamine But Not of GABA in Cultured Cerebellar Granule Cells

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The effects of arachidonic acid (20:4) on the uptake of glutamate were studied in primary cultures of cerebellar granule cells and were compared to cortical neurons and astrocytes. At a dose of 0.005 mM, the glutamate uptake was significantly inhibited in cerebellar granule cells. This inhibition was dose and time dependent. The uptake of glutamate was equally sensitive to 20:4 in primary cell cultures of cortical neurons, whereas the uptake in astrocytes was much less sensitive to 20:4. Glutamine uptake was inhibited by 20:4 in cultured cerebellar granule cells and cerebral cortical astrocytes but was not affected in cerebral cortical neurons. Furthermore, the uptake of gamma-aminobutyric acid was not affected by 20:4 in cerebellar granule cells.

Key words: astrocytes, glutamatergic neurons, GABA-ergic neurons, neurotransmitters

INTRODUCTION

Using brain slices and synaptosomal preparation Chan and his associates had demonstrated that arachidonic acid (20:4) was able to inhibit uptake of amino acid neurotransmitters glutamate and gamma-aminobutyric acid (GABA) [Chan et al, 1983]. Uptake inhibition of these amino acids in CNS is important because reuptake is the major mechanism for eliminating these extracellular accumulated neurotransmitters under normal physiological conditions [Johnson, 1978; Schousboe and Hertz, 1981]. Inhibition or malfunction of this reuptake system would lead to the accumulation of amino acids in the extracellular space. This is particularly critical in the case of glutamate and other excitatory neurotransmitters that have properties such as neuroexcitation [Johnson, 1978], depolarization [Bowman and Kimelberg, 1984] and edema induction [Van Harreveld and Fifkova, 1971; Møller et al, 1974; Chan et al, 1979].

We reported previously that 20:4 inhibited glutamate uptake in primary cultures of cerebral cortical astrocytes and neurons [Yu et al, 1986]. Results demonstrated that the uptake mechanism for glutamate in cerebral cortical neurons was more sensitive to 20:4 treatment than that of cerebral cortical astrocytes. In the case of GABA uptake, 20:4 only affected cerebral cortical neurons, whereas GABA uptake in astrocytes was not affected. The present investigation has been aimed at extending the study of 20:4 effects on cerebellar granule cells, a different type of neuronal culture, which is known to be glutamatergic in nature [Herndon and Coyle, 1977; Messer, 1977]. Thus, the purposes of these experiments are twofold. First, it is not clear whether the inhibitory effect of 20:4 on glutamate uptake is specific in cerebral cortex cultures; using cultures from cerebellum allows comparison of the 20:4 effect on uptake in cells of different origin. Second, the effect of 20:4 on glutamate and GABA uptake in both cerebellar granule cells (glutamatergic) and cerebral cortical neurons (GABAergic) can be compared. Furthermore, the effect of 20:4 on the uptake of glutamine is studied. This study is considered to be important because glutamine has no neurotransmitter properties but, metabolically, it is closely related to glutamate and GABA.

MATERIALS AND METHODS

Cell Cultures

Primary cultures enriched in cerebellar granule cells were prepared on the basis of the work by Messer [Messer, 1977], with minor modifications [Yu and Hertz, 1982a]. Cerebellar hemispheres of 7–8-day-old Sprague–Dawley rats were freed from the meninges, cut into small cubes, and trypsinized for 2–3 min with 0.2% trypsin in Puck's solution at room temperature. The trypsinized tissue was triturated with a 10-ml pipette. The resulting cell suspension was centrifuged for 5–10 min at 900g and

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pelleted cells were resuspended in a modified Eagle's minimum essential medium (MEM) [Hertz et al, 1982] containing an increased concentration of potassium (24.5 mM) and glucose (30 mM), as well as 0.1 mg/ml of p-aminobenzoic acid [Messer, 1977; Yu and Hertz, 1982a] and 5% horse serum (from Sterile System, Logan, UT). Tissue corresponding to one cerebellum per two dishes was seeded in 60-mm Falcon plastic tissue culture dishes precoated with D-polylysine [Yu and Hertz, 1982a; Yu et al, 1984]. After 15–20 min of incubation at 37°C, unattached cells (mostly nonneuronal cells) were removed together with the medium which was replaced with fresh medium. At day 4, the cultures were treated with 40 μM of cytosine arabinoside—for 24 hr to eliminate the proliferating astrocytes in the cultures. These cultures were used for uptake studies at day 21.

Primary cultures of cortical neurons were obtained from the brain hemispheres of 16–17-day-old Sprague-Dawley rat embryos and primary cultures of cortical astrocytes were prepared from newborn Sprague-Dawley rats. The procedure for the preparation of these cultures is described in previous publication [Yu et al, 1986].

Uptake of Amino Acids

Uptake of glutamate (50 μM), glutamine (500 μM), and GABA (50 μM) were determined as previously described [Yu and Hertz, 1982a; Yu et al, 1984, 1986]. For measurement of the effects of 20:4 on the uptake of these amino acids, cultures were washed two times with 37 °C serum-free modified MEM, then pre-incubated in medium containing the desired amount of 20:4 (0.005, 0.01, 0.05, and 0.1 mM). The preincubation times were 30 and 90 min. Corresponding control cultures had the same preincubation period of time but were not exposed to 20:4. At the end of the preincubation period, radioactive amino acid (0.1 μCi/ml) (Amersham, Arlington Heights, IL) was added directly to the culture. The uptake incubation lasted for exactly 5 min, a time needed to ensure that the uptake occurred at close to their initial rates [Yu and Hertz, 1982a]. In addition to this, the short uptake incubation time minimizes the loss of accumulated amino acid as CO_2, a metabolic process that may be quite pronounced in the case of glutamate [Yu and Hertz, 1982b]. After the incubation period, the cultures were rapidly washed twice with ice-cold MEM. One milliliter of 1 M NaOH was added and radioactivity and protein were determined in the dissolved cultures. Uptake rates at 5 min were calculated from the radioactivity per milligram of protein and the specific activity in the incubation media [Yu et al, 1984, 1986; Yu and Hertz, 1982a].

RESULTS AND DISCUSSION

Evidence from several lines of research have shown that cerebellar neuron-enriched cultures maintained in serum-containing medium are about 95% enriched by one particular neuronal cell population—the granule cells [Gallo et al, 1986]. These neurons are considered glutamatergic [Messer, 1977; Drejer et al, 1982]; therefore their cultures should be well suited to compare the differences in response to 20:4 of these neurons to GABAergic neurons obtained from cerebral cortex [Dichter, 1980; Yu and Hertz, 1982a; Yu et al, 1984, 1986]. Figure 1 shows cerebellar granule cells 21 days in culture. These neurons were cells attached to the polylysine-coated surface within the first 20 min of incubation. They can be kept in culture for over a month. Uptake of glutamate by the granule cells was at a rate of 4.92 nmole $\times$ mg^{-1} protein $\times$ min^{-1} and uptake of glutamine occurred at a rate of 1.85 nmole $\times$ mg^{-1} protein $\times$ min^{-1}, GABA was taken up at a lower rate of 0.73 nmole $\times$ mg^{-1} protein $\times$ min^{-1}. These findings were similar and in agreement with those reported in mouse cerebellar granule cell cultures by Yu and Hertz [1982a]. The uptake of GABA in this culture was very low, which agrees with others' findings that glutamatergic granule cells did not take up GABA [Messer, 1977; Drejer et al, 1982; Yu and Hertz, 1982a]. The development of rat cerebral astrocytes and neurons in culture has been previously reported [Yu et al, 1986].

Figure 2 shows that 20:4 inhibited the uptake of glutamate in cerebellar granule cells. Similar observation was reported in cerebral cortical neurons and cerebral astrocytes [Yu et al, 1986]. For 30-min treatment studies, 0.005 and 0.01 mM 20:4 did not affect the uptake, whereas at doses of 0.05 and 0.1 mM the uptake was significantly ($P < 0.0001$) inhibited. For 90 min studies, the glutamate uptake was inhibited significantly at a dose as low as 0.005 mM and an 80% inhibition was observed at 0.1 mM 20:4. This indicates that the effect of 20:4 on glutamate uptake is not only dose but is also time dependent. In comparison to cerebral cortical neurons, the uptake was equally sensitive to 20:4. As previously reported [Yu et al, 1986], astrocytic glutamate uptake was only inhibited at doses at least above 0.05 mM. Therefore, it is reasonable to believe that neurons, even from different origins and using different neurotransmitters, are more sensitive to 20:4 than astrocytes. Since the inhibition of glutamate uptake may have a detrimental effect on neuronal cellular swelling, this may also contribute to the greater vulnerability of neurons to edema inducing factors (eg, 20:4) than astrocytes.

Glutamine was taken up by cerebellar granule cells, astrocytes, and cerebral cortical neurons at a rate of 1.85 nmole $\times$ mg^{-1} protein $\times$ min^{-1}, 4.74 nmol $\times$ mg^{-1} protein $\times$ min^{-1} and 0.69 nmole $\times$ mg^{-1} protein $\times$ min^{-1}, respectively. The glutamine uptake, after a 90 min exposure to 0.1 mM 20:4, was inhibited significantly (40%) in cerebellar granule cells and in cerebral astrocytes (35%). The same concentration of 20:4 did not

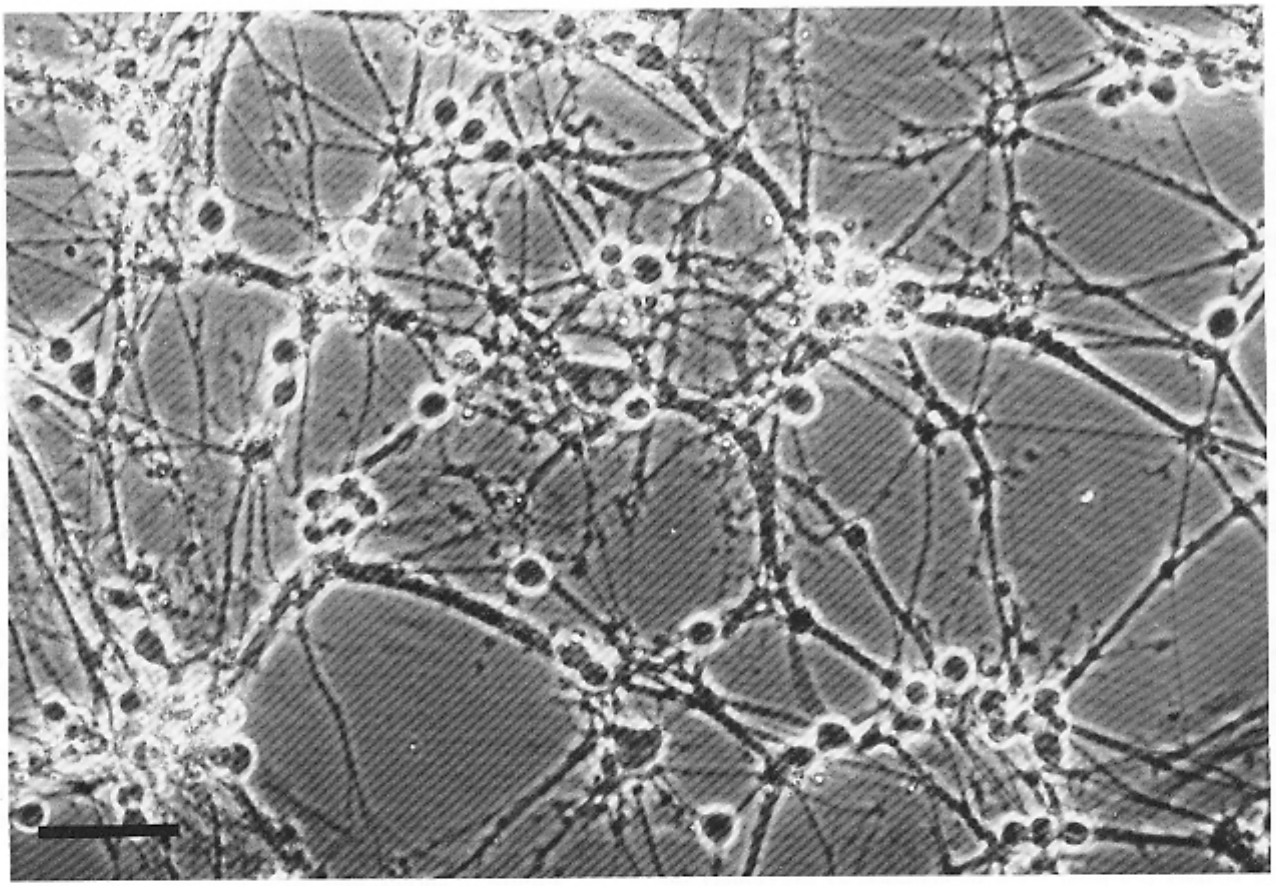

Fig. 1. Phase-contrast micrograph of primary culture of cerebellar granule cells. Cells were obtained from 7-day-old rats and grown for 21 days in culture with cytosine arabinoside treatment between day 4 and 5. Bar equals 40 μm.

affect glutamine uptake in cerebral cortical neurons. The reason for the lack of effect of 20:4 on glutamine uptake in cerebral cortical neuronal cultures is not clear at present. It may imply that these cells have different membrane properties than astrocytes and cerebellar granule cells. The above specificity was also observed in the case of GABA uptake.

GABA was taken up by all three types of cultures. Cerebral cortical neurons took up GABA at a higher rate, 1.5 nmole $\times$ mg^{-1} protein $\times$ min^{-1} [Yu et al, 1986],

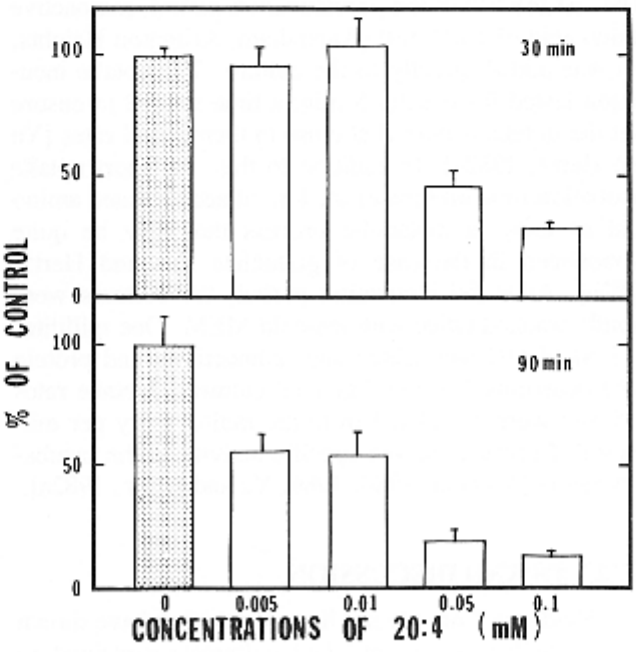

Fig. 2. Changes in the uptake rate of [^{14}C-U] glutamate, expressed as percentages of control values in primary culture of rat cerebellar granule cells after 30 (top) and 90 (bottom) min of exposure to various concentrations of 20:4. The uptake rates were measured at 5 min. The concentration of glutamate was 50 μM. Results are means of 4–6 experiments and SEM values are shown by vertical bars.

compared to astrocytes and granule cells, 0.13 nmol × mg^{-1} protein × min^{-1} and 0.73 nmole × mg^{-1} protein × min^{-1}, respectively; 0.1 mM of 20:4 affected GABA uptake in cerebral neurons [Yu et al, 1986], a GABAergic preparation [Dichter, 1980; Yu et al, 1984, 1986], but not in cerebellar granule cells and astrocytes as indicated in the present study.

It has been shown previously that the granule cells have a nonsaturable GABA uptake [Yu and Hertz, 1982a]. Thus, 20:4 may interfere with saturable neuronal GABA uptake but not with nonsaturable neuronal uptake. Based on these observations, the GABA uptake inhibition seen in brain slices [Chan et al 1983] may only be a GABAergic neuronal phenomenon, whereas the glutamate uptake inhibition in brain slices involved both neurons and astrocytes.

In summary, 20:4 affects glutamate uptake in cerebellar granule cells to the same extent as GABAergic cerebral cortical neurons, even though the neurons are cultured from different parts of the brain and utilize different neurotransmitters. The uptake of glutamate in neurons is more vulnerable to 20:4 than astrocytes. The effect of 20:4 on the uptake of amino acids (glutamine and GABA) in different types of cultured neural cells is very specific. This suggests that the inhibition is not due to a general alteration in membrane integrity caused by 20:4. Further studies are needed to elucidate the actual mechanisms and factors involved in the inhibitory effect of 20:4 on amino acid uptake.

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