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Amino acid neurotransmitters in the CNS

Relationships between net uptake and exchange in rat brain synaptosomes

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Carefully isolated, metabolically competent rat brain synaptosomes accumulate acidic amino acid neurotransmitters down to very low external levels. This supports the suggestion that nerve endings are involved in terminating transmission at the synapses and in maintaining low levels of these molecules in the external environment in the brain. At saturating levels of acidic amino acids, the rate of inward and outward movements of the Na^+-amino acid complex (exchange) is much faster than the net uptake. The transmembrane gradients of aspartate and glutamate approach each other under all conditions explored which indicates that these two amino acids share the same transport system.

Acidic amino acid transmitter; Neurotransmitter uptake; Neurotransmitter exchange; Synaptosome

1. INTRODUCTION

The discovery of high affinity, Na^+-dependent transport systems for amino acid neurotransmitters has led to the postulate that termination of transmission at synapses which utilize these molecules is accomplished by their reuptake into the terminals [1]. Later studies have thrown serious doubts on this hypothesis because at concentrations of amino acid transmitters close to their K_m values (4–20 μM) [2–4], the transporters were reported to mediate predominantly an exchange reaction and not net uptake [5]. We have observed however, that preparations of nerve ending particles (synaptosomes) incubated in media containing physiological [Na^+] and [K^+] were able to maintain very low external levels of amino acid neurotransmitters [6]: GABA < 1.0 μM; aspartate < 2 μM and glutamate 3–4 μM. Since these concentrations are very close to those found in the extracellular space of the brain in vivo [7–9], a reinvestigation of the relation between net uptake and exchange seemed of crucial importance for elucidating the mechanism of operation of amino acid transporters. Our results show that neurotransmitter amino acid transport systems are capable of net uptake down to external levels of amino acids much lower than their K_m values and hence are fully competent in terminating synaptic transmission.

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2. MATERIALS AND METHODS

Synaptosomes were isolated from the cortices and midbrains of 200–250 g male Sprague-Dawley rats as described in [10]. The final pellet was suspended in Krebs-Henseleit-Hepes buffer (140 mM NaCl, 5 mM KCl, 10 mM Tris-Hepes, 5 mM $NaHCO_3$, 1.3 mM $MgSO_4$ and 1 mM NaH_2PO_4), pH 7.4, and supplemented with 10 mM glucose and 1.27 mM $CaCl_2$. All incubations were carried out at 30°C in a shaking water bath.

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trasynaptosomal [K^+] were measured as in [11]. The levels of ATP, creatine phosphate and creatine were determined by standard enzymatic procedures [12,13]. Protein was measured by the biuret reaction [14].

2.1. *Measurements of net fluxes of aspartate and glutamate*

Synaptosomes (17–20 mg protein/ml) were preincubated for 10 min before being diluted 20-fold into media containing either normal or altered concentrations of sodium and potassium and supplemented either with 20 or 70 μM glutamate (or aspartate). Samples (250 μl) were withdrawn after 7, 15 and 30 min incubation for the determinations of intra- and extrasynaptosomal aspartate and glutamate by high-pressure liquid chromatography (HPLC) as described in [15]. Aliquots were also removed and diluted 20-fold into the medium containing no added glutamate (or aspartate) for measurements of the levels of amino acid before addition of glutamate (aspartate).

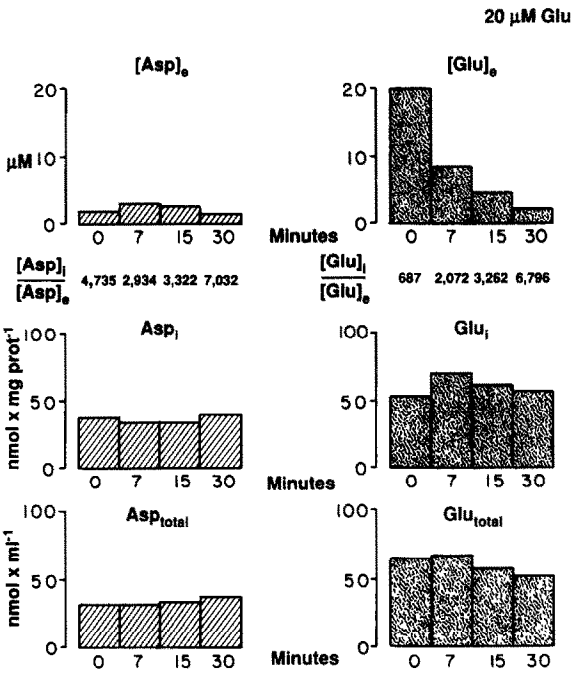

Fig.1. Effect of addition of 20 μM glutamate on transmembrane movements of aspartate and glutamate. Transmembrane fluxes of aspartate and glutamate were measured as described in section 2. Protein concentration was 0.85 mg/ml. One of three experiments is presented.

3. RESULTS

3.1. *General characteristics of the synaptosomal preparations*

In preliminary experiments, the quality of synaptosomal preparations was evaluated by measuring the concentrations of high-energy phosphate compounds and the levels of intrasynaptosomal potassium. ATP was present at 3.73 ± 0.51 nmol/mg protein, creatine phosphate at 6.42 ± 0.57 nmol/mg protein and creatine at 18.67 ± 2.25 nmol/mg protein (means ± SD, n = 3). At an external potassium concentration of 4.3 ± 0.18 mM, the intrasynaptosomal K^+ was found to be 257 ± 11 nmol/mg protein (mean ± SD, n = 7) or 64.3 mM (intrasynaptosomal water space was found to be 4 ± 0.5 μl/mg protein); this gives a value of 15 for the $[K^+]_i/[K^+]_e$. In the absence of added amino acids, mean values for intrasynaptosomal aspartate and glutamate were 27.3 ± 5 and 50 ± 4 nmol/mg protein, respectively (means ± SD, n = 7).

3.2. *The effect of external glutamate on transmembrane amino acid fluxes at physiological [Na^+] and [K^+]*

Incubations of synaptosomes with externally added glutamate resulted in a rapid increase in the level of this amino acid inside the synaptosomes and a parallel decrease in its concentration in the medium (figs 1,2). It can be seen that with external glutamate of either 20 or 70 μM internal glutamate rose from about 54 to about 70 nmol/mg protein during the first 7 min while its external concentration fell from 20 to 8 μM and from 70 to 46 μM, respectively. The inward movement of glutamate was accompanied by an efflux of aspartate which was greater at the higher external glutamate level. Moreover, at the time when glutamate was added (time '0') the $[glutamate]_i/[glutamate]_e$ ratios were markedly lower (687 and 195 for the lower and higher added glutamate, respectively) than the $[aspartate]_i/[aspartate]_e$ (about 4000 for both conditions), yet after 7 min incubation they were the same, within experimental error, in samples with 20 μM added glutamate and only by a factor of 3

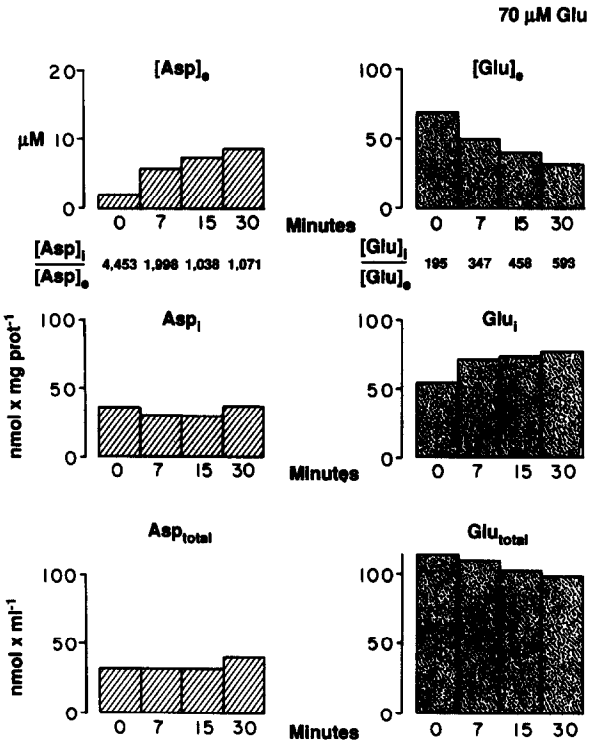

Fig.2. Effect of addition of 70 μM glutamate on transmembrane movements of aspartate and glutamate. Conditions are those given in the legend to fig.1.

smaller in incubates with 70 μM glutamate. During the next 23 min incubation, further albeit small changes were observed. External glutamate continued to decrease while [aspartate]$_e$ gradually declined in samples with added low glutamate but increased in those with higher added glutamate. Intrasynaptosomal aspartate increased in both conditions. It is important to point out that with time, total glutamate (i.e. external plus intrasynaptosomal) decreased while total aspartate increased by approximately the same amount. This suggests that alterations in metabolism of the two amino acids are superimposed on changes in their fluxes and that these metabolic readjustments may affect the internal and external levels of these molecules. Figs 1 and 2 show, however, that whatever the nature of these metabolic alterations, they do not influence the overall behavior of the system: the accumulation ratios of glutamate and aspartate approach the same value and once they reach it, they both change in concert.

3.3. *The effect of alterations in [K^+] and [Na^+] on glutamate and aspartate movements*

Movements of aspartate and glutamate across the synaptosomal membrane were also investigated when glutamate was added to media containing altered potassium and sodium concentrations. When $[K^+]_e$ was raised to 10 mM, the pattern of changes which followed addition of 20 μM glutamate was similar to that shown in fig.1 for 5 mM $[K^+]_e$: the concentration of glutamate in the medium decreased whereas that of aspartate increased. This was accompanied by opposite changes in the intrasynaptosomal levels of the two amino acids (fig.3). Quantitatively, however, the uptake of glutamate was smaller while the efflux of aspartate was larger. Consequently the equilibrated values of [amino acid]$_i$/[amino acid]$_e$ were 2–3-fold lower than those at 5 mM $[K^+]_e$. In spite of the decrease in the absolute values for amino acid gradients, but consistent with the results in fig.1, the [glutamate]$_i$/[glutamate]$_e$ became equal, within the limits of experimental error, to the [aspartate]$_i$/[aspartate]$_e$. When the experiments were carried out with 70 μM glutamate, the results were essentially identical to those shown in fig.2, in spite of a higher external potassium concentration (not shown).

The pattern of changes which follow addition of 20 μM glutamate to the medium containing 30 mM K^+ is shown in fig.3B. It can be seen that under these conditions both glutamate and aspartate were released into the medium, the efflux of the latter being, on a percentage basis, greater than that of the former. Again, after 7–15 min incubation the gradients of the two amino acids approached each other, within a factor of 2.

When synaptosomes were diluted into the medium containing 30 mM K^+ and 70 μM glutamate, there was a small uptake of glutamate reducing the external level to about 62 μM within 7 min and a large release of aspartate to give an external concentration of 10–14 μM. After 15 min incubation the [glutamate]$_i$/[glutamate]$_e$ gradients were about 200 and those for aspartate were about 400 (not shown).

The effect of lowered $[Na^+]$ in the medium is illustrated in fig.4. It can be seen that when the con-

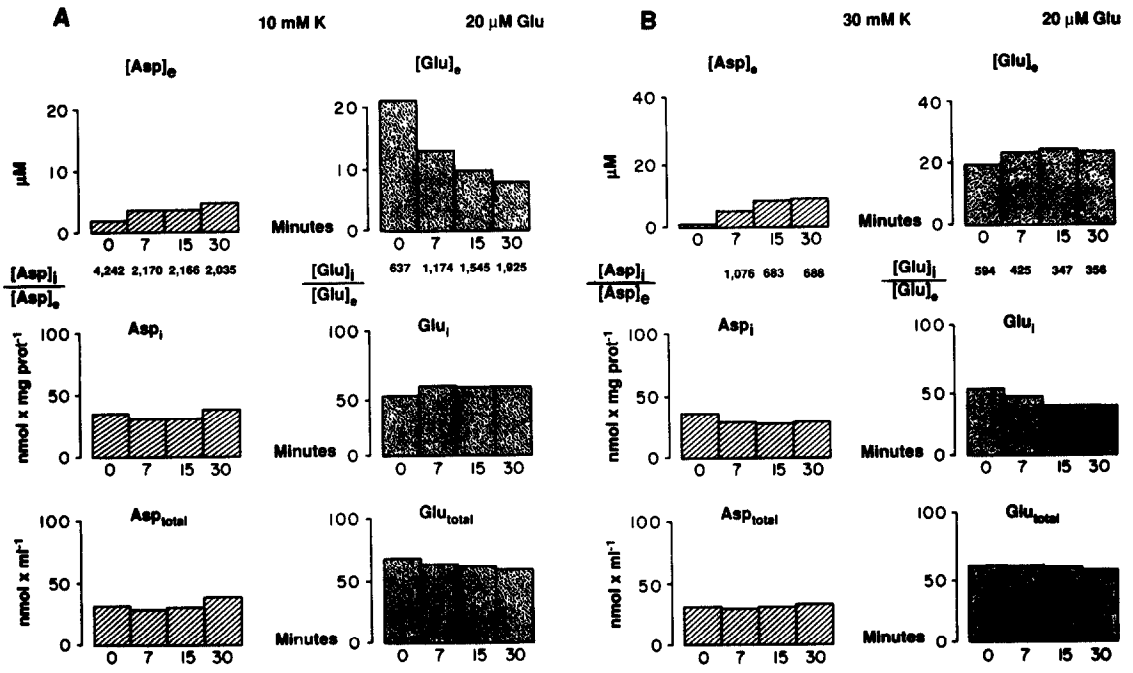

Fig.3. Effect of K^+ on transmembrane movements of acidic amino acid transmitters. Transmembrane fluxes of aspartate and glutamate were measured as described in section 2 after 20-fold dilution of synaptosomes into the medium containing final $[K^+]_e$ of 10 mM (A) or 30 mM (B). Protein concentration was 0.85 mg/ml. One of three experiments is presented.

centration of sodium was reduced to 15 mM, addition of 20 μM glutamate was followed by a net release of this amino acid. This was accompanied by a parallel and large efflux of aspartate from synaptosomes into the medium.

3.4. *The effect of aspartate on acidic amino acid fluxes*

Experiments were also carried out in which synaptosomes were diluted into media of essentially the same composition as those described above but containing either 20 or 70 μM aspartate instead of glutamate. Under such conditions, uptake of aspartate from the medium was accompanied by a release of glutamate from synaptosomes, to an extent that the gradients of the two amino acids became equal, within a factor of 2. The responses to different concentrations of aspartate and to altered $[Na^+]_e$ and $[K^+]_e$ were analogous to those observed for glutamate addition (not shown).

4. DISCUSSION

The present results demonstrate that carefully isolated rat brain synaptosomes, in which the energetic parameters and the internal content of potassium are high, are able to accumulate acidic amino acid neurotransmitters down to very low external levels. This finding supports the previous suggestion [1] that nerve endings themselves are involved in terminating transmission at the synapses and in maintaining low levels of neurotransmitters in the external environment of the brain.

Our data also show that net movements of acidic amino acid transmitters are dependent on two factors: external concentrations of sodium and potassium on the one hand, and of the amino acids themselves, on the other. The former is consistent with the earlier postulate from this [15,16] and other [17,18] laboratories that the uptake of amino acid transmitters is fueled by a combination of the transmembrane electrical potential, i.e.

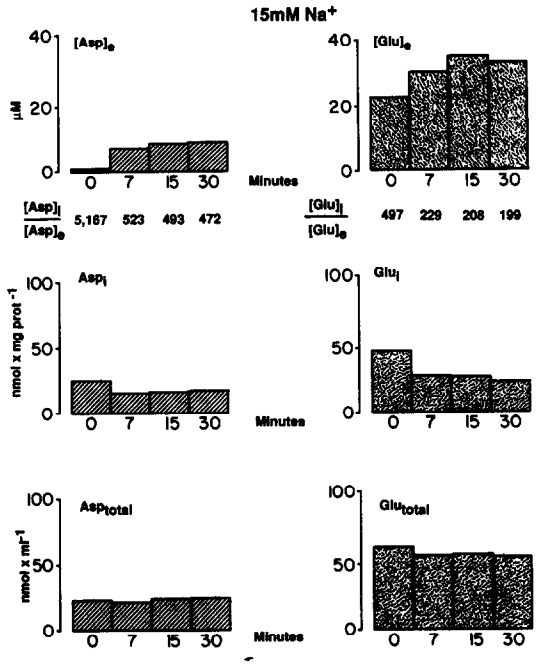

Fig.4. Effect of Na^+ on transmembrane movements of aspartate and glutamate. Synaptosomes were diluted 20-fold into the medium in which NaCl was replaced completely by choline chloride. Final $[Na^+]$ was determined to be 15 mM. Glutamate was added at 20 μM. Amino acid fluxes were measured as given in section 2. Protein concentration was 0.89 mg/ml. One of four experiments is presented.

$[K^+]_i/[K^+]_e$ and the sodium concentration gradient. The greater the magnitude of the driving forces, the larger the transmembrane gradients of the amino acids that can be generated and maintained. It can be calculated from this and our previous work [19] that in synaptosomes suspended at physiological sodium and potassium concentrations the $[K^+]_i/[K^+]_e$ is about 15 and the $[Na^+]_e/[Na^+]_i$ about 5.4. Since the uptake of each acidic amino acid molecule occurs in cotransport with 2 Na^+ [15] the maximum accumulation ratio of aspartate (or glutamate) that is permitted by the combined driving forces is $15^2 \times 5.4^2$, i.e. 6561, a value within the limits of experimental error equal to the magnitudes of the amino acid gradients measured in the same conditions (see the figures). When the driving forces are decreased, either due to a rise in $[K^+]_e$ or a fall in $[Na^+]_e$, the gradients of the amino acids that can be maintained must decline appropriately. Hence, if synaptosomes preincubated at high external sodium and low external potassium are diluted into media containing low $[Na^+]_e$ and high $[K^+]_e$, net efflux of amino acids is observed until a near equilibrium with the new driving forces is attained. If the external concentration of either glutamate or aspartate is then raised, a net uptake is observed because the driving forces temporarily exceed the amino acid accumulation ratio. This behavior of the amino acid transporters indicates that the systems are readily reversible and that during action potential-induced depolarization of nerve terminals they will mediate net release of amino acid transmitters. However, as soon as the ionic gradients are restored by the ion pumps the same systems will revert to operating in the direction of net uptake which will then occur at maximal velocity in the presence of high extracellular levels of these molecules.

There are two other interesting observations which deserve comment. The first is that at high external glutamate concentrations, i.e. $>K_m$, a large and rapid release of aspartate was observed despite the continued presence of high driving forces. This means that at saturating levels of substrates the transporters mediate a rapid exchange reaction [20–23] and that the rates of the inward and the outward movements of the 2 Na^+-amino acid complex are, under these conditions, much faster than the net uptake. Such behavior is to be expected from a reversible cotransport system [24].

The second observation concerns the near equality of the transmembrane gradients of glutamate and aspartate under all conditions investigated in this study. Such behavior means that acidic amino acids share the same transport system for their movements across the synaptosomal membrane. This conclusion is consistent with the known cross-competition for uptake between the two amino acids [25–27] and the ability of one to stimulate release of the other (heteroexchange [20–23]).

ACKNOWLEDGEMENT

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