Transamination of glutamate to tricarboxylic acid-cycle intermediates in cultured neurons correlates with the ability of oxo acids to support neuronal survival *in vitro*

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Cultures of central-nervous-system neurons at low densities require for their survival exogenous pyruvate, α -oxoglutarate or oxaloacetate, even in the presence of high glucose concentrations. Most other α -oxo acids support cell survival only in the presence of α -amino acids which transaminate to α -oxoglutarate, oxaloacetate or pyruvate. The α -oxo acids therefore operate as acceptors of amino groups from appropriate donors to generate tricarboxylic acid-cycle-relevant substrates, and these α -oxo acids provide for neuronal support only insofar as they make it possible for exogenously supplied α -amino acid precursors to generate intracellularly one of the three critical metabolites. To examine more closely the relationship between transamination activity and neuronal survival, we measured $^{14}\text{CO}_2$ production from [^{14}C]glutamate in the presence of appropriate α -oxo acid partners by using 8-day-embryonic chick forebrain, dorsal-root-ganglion and ciliary-ganglion neurons. Neuronal survival was measured concurrently in monolayer neuronal cultures maintained with the corresponding amino acid/oxo acid pairs. Forebrain and ganglionic cell suspensions both produced $^{14}\text{CO}_2$ from [^{14}C]glutamate, which accurately correlated with 24 h neuronal survival. Concentrations of glutamate or α -oxo acid which provide for maximal neuronal survival also produced maximal amounts of $^{14}\text{CO}_2$. The same ability to generate CO_2 from glutamate (in the presence of the appropriate α -oxo acids) can ensure neuronal survival in 24 h cultures and therefore must meet energy or other metabolic needs of those neurons which glucose itself is unable to satisfy.

INTRODUCTION

Pyruvate and other selected α -oxo acids have long been identified as enhancing the growth of animal cells cultured in the presence of glucose (Newman & McCoy, 1958; McKeehan & McKeehan, 1979; Sens *et al.*, 1982). We have shown that CNS neurons, seeded at low densities in a serum-free medium, require pyruvate for their short-term (24 h) survival (Selak *et al.*, 1985). We have also found that (i) pyruvate can be replaced by oxaloacetate and α -oxoglutarate, and none of these three α -oxo acids requires the concurrent presence of transamination partners, (ii) selected amino acids, capable of generating pyruvate, oxaloacetate or α -oxoglutarate, can also permit neuronal survival, but only if accompanied by α -oxo acids serving as transamination acceptors (Facci *et al.*, 1985a), and (iii) the amino acid-independent and the amino acid-requiring α -oxo acids can be equally effective at 8-fold lower concentrations when accompanied by certain other low- M_r agents (unable by themselves to secure neuronal survival) (Facci *et al.*, 1985b). The basis for this neuronal dependence on exogenous pyruvate or pyruvate surrogates and the way by which pyruvate-sparing agents achieve their effect are questions that remain to be addressed.

Pyruvate may be needed by the cultured CNS neurons for some aspect of their energy metabolism. One parameter used to monitor energy-related metabolism is the rate of $^{14}\text{CO}_2$ formation from radioactive energy precursors supplied to the neurons *in vitro*. In a previous

study, transamination reactions were indeed shown to be involved when α -oxo acids were used to support neuronal survival instead of pyruvate (e.g., Facci *et al.*, 1985a). In the present experiments we show that:

- (i) CNS and PNS cell suspensions display an ability to produce $^{14}\text{CO}_2$ from [^{14}C]glutamate which precisely correlates with 24 h neuronal survival;
- (ii) the ability of all these neurons to generate CO_2 from glutamate reflects intrinsic properties;
- (iii) the same ability to generate CO_2 from glutamate (in the presence of appropriate α -oxo acids) can ensure neuronal survival in 24 h cultures.

EXPERIMENTAL

Materials

Eagle's basal medium (EBM) was from Grand Island Biological Co., Grand Island, NY, U.S.A. Human transferrin, bovine insulin, progesterone, putrescine, polyornithine, Hepes, oxo acids, amino acids and ovalbumin were from Sigma Chemical Co., St. Louis, MO, U.S.A. L-[U- ^{14}C]Glutamic acid (225 Ci/mol) was from ICN, Irvine, CA, U.S.A. All other chemicals were of the highest purity available.

Cell systems

EBM, modified to contain 33.3 mM-D-glucose, 100 units of penicillin/ml and 20 mM-Hepes, and supplemented with the N1 mixture (0.83 μM -insulin, 62 nM-

Abbreviations used: CNS, central nervous system; PNS, peripheral nervous system; EBM, Eagle's basal medium; ED_{50} , effective concentration giving a half-maximal response.

transferrin, 0.1 mM-putrescine, 20 nM-progesterone and 30 nM- Na_2SeO_3) (Skaper *et al.*, 1979), containing no glutamine and with or without bicarbonate supplement (EBM/N1/Hepes), was used throughout as the incubation medium, with various additions as indicated. Cell suspensions were prepared from 8-day embryonic chick forebrain by mechanically dispersing the cells (after trypsin treatment of the tissue) in incubation medium containing 1% (w/v) ovalbumin, and then diluting them with albumin-free medium to the desired cell densities (Varon *et al.*, 1984).

In the standard culture system, 6 mm wells of a micro-test plate (Costar) were coated with polyornithine and RN22 schwannoma neurite-promoting factor (Manthorpe *et al.*, 1981), supplied with 50 μl of EBM/N1/Hepes (supplemented to 26.4 mM- NaHCO_3) containing test agent, and then seeded with 50 μl of cell suspension diluted to 20000 cells/ml in the same (but agent-free) medium. The cultures were fixed with 2% (v/v) glutaraldehyde in EBM after 24 h of incubation (at 37 °C, under 5% CO_2 in air), and neurons were counted under phase microscopy over two 0.5 mm $\times$ 6 mm strips. Additionally, two other culture systems were employed: (i) monolayer neuronal cultures in stoppered T25 (25 cm²) flasks (Costar), also coated with polyornithine and schwannoma neurite-promoting factor, with NaHCO_3 supplementation of the medium before introduction of radioactive substrate and without it during labelling; (ii) suspension cultures in stoppered tubes (sterile, polyethylene, 13 mm $\times$ 100 mm) in EBM/N1/Hepes with no bicarbonate supplement.

¹⁴CO₂ measurements

Cells were introduced into tubes or T25 flasks. The vessels, immediately before introduction of radioactive substrate, were capped with rubber stoppers carrying hanging wells, in which a multifolded filter paper (4 cm $\times$ 1 cm) had been placed and 0.2 ml of 1 M-NaOH added to the paper just before capping (Skaper *et al.*, 1976). Tubes were placed in a reciprocal shaker and flasks in a culture (air) incubator at 37 °C. At the chosen times, L-[U-¹⁴C]glutamic acid (constant specific radioactivity in all experiments) and the appropriate non-radioactive α -oxo acid were added, and incubation was continued for the desired 'pulse' time. Then 2.5 M- H_2SO_4 was introduced through the stopper and, after another 60 min incubation, the centre wells were retrieved, placed into 4.5 ml of scintillation fluid, and counted for radioactivity. The procedure is designed to trap all the CO_2 produced during the incubation into the alkali-impregnated filter paper, and measure the ¹⁴CO₂ output derived from the ¹⁴C-labelled source (here glutamate). With the T flasks, moreover, the number of surviving neurons can be determined before and after the radioisotope pulse (but before the H_2SO_4 introduction). Details on volumes, concentrations and incubation times are given where first used.

RESULTS

The T25-flask monolayer-culture system

The standard 6 mm microwell has a surface of about 28 mm², and 1000 forebrain cells seeded in 0.1 ml of trophic (survival-supporting) medium will lead to the 24 h survival of about 500 neurons. The proportional

Fig. 1. Production of ¹⁴CO₂ and neuronal survival as a function of cell number seeded for 8-day-embryonic chick forebrain cells

T25 flasks were seeded with different numbers of cells in 4 ml of EBM/N1/Hepes without (○) or with (●) 2 mM-L-glutamate and 1 mM-phenylpyruvate. At 19 h, glutamate/phenylpyruvate-support cultures received 2 ml of fresh medium containing 2 μCi of L-[U-¹⁴C]glutamate/ml and 2 mM-L-glutamate plus 1 mM-phenylpyruvate. Release of ¹⁴CO₂ was measured over a 5 h incubation period (open bars).

values for a T25 flask (with a 2500 mm² area) would be 90000 cells seeded in 9 ml of medium. Increasing the cell seeding density (and presumably also decreasing the volume of medium) will favour self-supported neuronal survival (Varon *et al.*, 1984), thereby decreasing the opportunities to analyse exogenous trophic agents. We therefore chose to examine flask cultures in which different cell numbers were seeded with the minimal usable volume of 4 ml medium. The cultures were pulsed with 2 ml of EBM/N1/Hepes containing 2 mM-L-[U-¹⁴C]glutamate (2 μCi /ml) and 1 mM-3-phenylpyruvate between 19 and 24 h of culture time, just before being fixed. The results, illustrated in Fig. 1, established several points.

(a) In cultures supported by glutamate/phenylpyruvate, neuronal survival was proportional to the number of cells seeded at about 60% of the seed (a better survival than the 50% in 6 mm microwells).

(b) Glutamate/phenylpyruvate-free cultures allowed no survival at 10⁵ cells seeded/flask, but started displaying some independent survival already at twice (2 $\times$ 10⁵) that value (the microwell system, using twice the medium-volume/cell-seed ratio, required at least a 5-fold increase over the standard seedings; cf. Varon *et al.*, 1984).

(c) At 10⁵ cells seeded/flask with glutamate/phenylpyruvate, ¹⁴CO₂ production was about 1500 d.p.m. per 5 h pulse.

We concluded from these initial experiments that an acceptable system could be achieved by the above conditions, namely seeding 10⁶ cells/flask in 4 ml and pulsing for 5 h with 2 ml of medium containing radioactive substrate at 2 μCi /ml. Unless stated otherwise, these conditions apply to all subsequent experiments.

Table 1. Comparison of $^{14}\text{CO}_2$ production from CNS cell suspensions and neuronal monolayer cultures

Suspensions contained 10^5 8-day-embryonic chick forebrain cells in 0.2 ml of EBM/N1/Hepes, and T25 flasks contained 10^5 cells in 4 ml of EBM/N1/Hepes without ('2–7 h') or with ('19–24 h') 2 mM-glutamate and 1 mM-phenylpyruvate (PhePyr). After 2 h, suspensions received 0.16 ml and 2–7 h flasks 2 ml of medium containing the indicated concentrations of L-[U- ^{14}C]glutamate and phenylpyruvate. The 19–24 h flasks were washed (at 19 h) with medium, and given 2 ml of medium with substrates. Sparing agents, where present, consisted of 0.4 mM-serine, 0.02 mM-pyridoxal, 0.48 mM-arginine, 0.25 mM-cystine, 0.80 mM-leucine, 0.80 mM-isoleucine, 0.08 mM-tryptophan and 0.57 mM-tyrosine. Results are means $\pm$ S.D. ($n = 6$).

Culture . . . Pulse interval (5 h) . . .	$^{14}\text{CO}_2$ production (d.p.m./5 h per culture)		
	T25 flask 19–24	T25 flask 2–7	Suspension 2–7
2 mM-Glu + 1 mM-PhePyr	1921 $\pm$ 188	1951 $\pm$ 336	1785 $\pm$ 170
2 mM-Glu + 0.08 mM-PhePyr	40 $\pm$ 10	20 $\pm$ 10	50 $\pm$ 20
0.25 mM-Glu–1 mM- PhePyr	30 $\pm$ 10	40 $\pm$ 10	40 $\pm$ 15
0.25 mM-Glu + 0.08 mM-PhePyr + 'sparing' agents	1740 $\pm$ 200	1850 $\pm$ 110	1696 $\pm$ 155

Equivalent requirements for $^{14}\text{CO}_2$ production and neuronal survival

Samples (10^5 cells) from the same chick forebrain dissociated preparation were seeded into T25 flasks with 4 ml of medium containing 2 mM-glutamate and 1 mM-

phenylpyruvate, or delivered into 13 mm $\times$ 100 mm polyethylene tubes with 0.2 ml of the same medium. All cultures were pulsed for 5 h by addition of [^{14}C]glutamate, for a final concentration of 2 $\mu\text{Ci}/\text{ml}$, to (i) 19 h flask cultures, (ii) 2 h flask cultures and (iii) 2 h tube suspensions. The results, illustrated in Table 1, demonstrate the following.

(a) Freshly attached neurons (2–7 h-pulsed flasks), still largely lacking neurites, generated $^{14}\text{CO}_2$ to the same extent as did those cultured in monolayer for about 1 day (19–24 h-pulsed flasks), the time at which neuronal survival and its requirements have been most studied. Thus any survival requirements that would be reflected in the $^{14}\text{CO}_2$ production do not develop because of time in culture.

(b) The same $^{14}\text{CO}_2$ production was observed by neurons incubated for the same time period in dispersed monolayer cultures and in loosely clustered cell suspensions (2–7 h-pulsed flasks and tubes, respectively). Therefore, attachment to and interaction with the culture substratum do not ostensibly modify $^{14}\text{CO}_2$ production. Note also that, in the tube, the cells were not only clustered but confined to less than 0.4 ml of total fluid, compared with the 4 ml of the flasks. Nor was the $^{14}\text{CO}_2$ production influenced by the volume of fluid to which the 10^5 cells were exposed during radioactive pulsing: less than 0.4 ml in the tubes, 2 ml in the flask.

(c) In both suspensions and monolayers and at both earlier and later culture times, practically no $^{14}\text{CO}_2$ was released if either the α -oxo acid receptor or the α -amino acid donor concentration was decreased 10-fold, as previously observed with 24 h neuronal survival (Facci *et al.*, 1985a). Unlabelled α -oxoglutarate (2 mM) inhibited $^{14}\text{CO}_2$ release from [^{14}C]glutamate by more than 50% (results not shown).

(d) We have previously shown that the pyruvate concentrations required by 8-day-embryonic chick forebrain neurons for survival are decreased substantially by

Table 2. Evaluation of the α -oxo acid requirement specificity by $^{14}\text{CO}_2$ production from cell suspensions and by 24 h neuronal survival for 8-day-embryonic chick forebrain

Each tube contained 10^5 forebrain cells in 0.36 ml of EBM/N1/Hepes, 2 mM-L-[U- ^{14}C]glutamate (2 $\mu\text{Ci}/\text{ml}$) and 1 mM-oxo acid as indicated, and $^{14}\text{CO}_2$ production was measured between 2 and 7 h. Microwells (6 mm) contained 1000 cells in 0.1 ml of EBM/N1/Hepes plus 2 mM-glutamate and 1 mM-oxo acid, and survival was measured at 24 h. Results are means $\pm$ S.D. ($n = 6$).

Oxo acid added	$^{14}\text{CO}_2$ production (d.p.m./5 h per tube)	Neuronal survival (%)
None	0	0
Phenylpyruvate	1645 $\pm$ 145	47
4-Methyl-2-oxopentanoate	1650 $\pm$ 110	50
2-Oxobutyrate	1688 $\pm$ 98	49
3-Methyl-2-oxobutyrate	1598 $\pm$ 104	49
2-Oxopentanoate	1433 $\pm$ 128	52
3-Methyl-2-oxopentanoate	1493 $\pm$ 90	51
2-Oxoadipate	1392 $\pm$ 105	50
2-Oxomalonate	1676 $\pm$ 66	51
2-Oxo-octanoate	0	0
2-Oxoglutarate	0	0
3-Oxoglutarate	0	0
3-Oxoadipate	0	0
Acetoacetate	0	0
Laevulinate (4-oxopentanoate)	0	0

other low- M_r agents present in Dulbecco's modified Eagle's medium (Selak *et al.*, 1985). These molecules consist of an obligatory mixture of serine, pyridoxal, arginine, cystine, leucine, isoleucine and tryptophan (Facci *et al.*, 1985b). As Table 1 (last line) also shows, low concentrations of glutamate and phenylpyruvate can lead to the same high $^{14}\text{CO}_2$ release as do their high concentrations, if the medium is also supplemented with the above 'sparing' agents identified with chick forebrain neuronal survival. As before, all three culture systems behaved comparably.

Table 2 shows that the requirement for an α -oxo acid to complement glutamate showed the same specificities whether evaluated by $^{14}\text{CO}_2$ release in early cell suspensions or by 24 h neuronal survival in monolayer cultures. Most α -oxo acids, but not α -oxo-octanoate or -nonanoate, were competent for both functions, whereas β - or γ -oxo acids were competent for neither.

We may therefore conclude that: (i) freshly prepared forebrain cell suspensions generate $^{14}\text{CO}_2$ from

^{14}C glutamate to about the same extent and with apparently the same substrate restrictions as they do in early or 24 h-old monolayer neuronal cultures, and (ii) substrate restrictions for $^{14}\text{CO}_2$ production parallel those previously identified (Facci *et al.*, 1985a) for the 24 h survival of cultured CNS neurons.

Quantitative comparisons between $^{14}\text{CO}_2$ production and survival requirements

The qualitative equivalence between the requirements displayed by each of these two performances has been taken to a quantitative level and extended to other known features of neuronal regulation in the following experiments.

Glutamate and appropriate α -oxo acids were individu-

Fig. 2. Comparison of $^{14}\text{CO}_2$ production and quantitative survival requirements for 8-day-embryonic chick forebrain neurons

Samples of forebrain cells ($10^5/0.36$ ml) were pulsed with L-[U- ^{14}C]glutamate between 2 and 7 h, and other samples (1000 cells/0.1 ml) were cultured for 24 h in microwells. (a) Different concentrations of glutamate (constant specific radioactivity) were titrated against 1 mM-phenylpyruvate without (●, ○) or with (■, □) CNS-sparing agents (see Table 1). (b) Different concentrations of phenylpyruvate (●, ○, ■, □) or 2-oxopentanoate (▲, △, ◆, ◇) were titrated against 2 mM-glutamate, again without (●, ○, ▲, △) or with (■, □, ◆, ◇) sparing agents. Closed symbols show 24 h neuron survival, and open symbols $^{14}\text{CO}_2$ production. Values are averaged from two experiments.

Fig. 3. Concentration-dependence on glutamate of PNS neurons assessed by $^{14}\text{CO}_2$ production and neuronal survival

Samples of purified 8-day-embryonic chick ciliary-ganglion or dorsal-root-ganglion neurons (35000 neurons/0.36 ml), prepared as described by Manthorpe *et al.* (1983) were pulsed with L-[U- ^{14}C]glutamate between 2 and 7 h, and other samples (1000 neurons/0.1 ml) were cultured for 24 h in microwells. Ciliary-ganglion neuron cultures were supplemented with 100 trophic units of chick eye-derived Ciliary Neurotrophic Factor (Barbin *et al.*, 1984)/ml, and dorsal-root-ganglion neuron cultures with 100 trophic units of mouse submaxillary Nerve Growth Factor (Varon *et al.*, 1972)/ml. Phenylpyruvate was at 1 mM throughout; when added serine was at 0.4 mM and Fe^{3+} at $6.5 \mu\text{M}$. Control (●, ○); plus serine (■, □); plus serine and Fe^{3+} (▲, △). Closed symbols show neuronal survival, and open symbols $^{14}\text{CO}_2$ production. Values are averaged from two experiments.

ally titrated in the constant presence of optimal amounts of the other partner. Samples of a dissociated forebrain preparation were pulsed in suspension with [^{14}C]glutamate between 2 and 7 h, and other samples of the same preparations were cultured for 24 h-survival measurements in 6 mm microwells. In addition, and for both determinations, replicate samples used medium supplemented with the previously identified CNS-sparing agents (see Facci *et al.*, 1985b and Table 1). Fig. 2 shows that early $^{14}\text{CO}_2$ production by the cell suspensions paralleled the quantitative requirements of later neuronal survival in monolayer cultures. Specifically: (i) the same concentrations giving a half-maximal response (ED_{50}) were found with the two determinations for both glutamate (970 μM for control, 120 μM with sparing agents) (Fig. 2a) and the required α -oxo acids (300 μM and 40 μM respectively) (Fig. 2b) and the same ED_{50} changes were produced in all cases by the concurrent use of sparing agents, and (ii) maximal $^{14}\text{CO}_2$ production was constant whenever maximal survival was sustained, regardless of excess glutamate, α -oxo acid or both, suggesting that under these circumstances $^{14}\text{CO}_2$ measures the 'viability' of the cell populations.

A second approach was to compare early $^{14}\text{CO}_2$ production and later neuronal survival by using purified PNS neuronal preparations previously identified for their partial dependence on pyruvate (Skaper *et al.*, 1984). Fig. 3(a) demonstrates that 8-day-embryonic chick ciliary ganglion neurons (i) display the same dose-dependence on glutamate (ED_{50} 400 μM) (in the constant presence of 1 mM-phenylpyruvate) whether assessed by early $^{14}\text{CO}_2$ performances or later neuronal survival, and (ii) decrease their ED_{50} for glutamate in exactly the same way for both properties when supplemented with appropriate sparing agents such as serine (ED_{50} 42 μM) or serine and Fe^{3+} ions (ED_{50} 12 μM) (Skaper *et al.*, 1984). The same conclusions can be derived with 8-day-embryonic chick dorsal root ganglion neurons, as shown in Fig. 3(b), for which serine alone serves as a sparing agent (Facci *et al.*, 1985a). In this case, the ED_{50} for glutamate was 330 μM in the absence of serine, and 180 μM in its presence. Similar observations were made for these peripheral neurons when phenylpyruvate was titrated with a constant concentration of glutamate (results not shown).

DISCUSSION

We have established here that a neuronal cell suspension displays, shortly after tissue dissociation, an ability to produce $^{14}\text{CO}_2$ from [^{14}C]glutamate which precisely correlates with 24 h neuronal survival in culture, with respect to: (i) the requirement for concurrent availability of the appropriate α -oxo acids, (ii) the ED_{50} for both glutamate and α -oxo acids, (iii) the ED_{50} sensitivities to the appropriate sparing agents, and (iv) the applicability of all those features to PNS as well as CNS neurons. Several conclusions can be drawn from these findings:

(a) The ability of all these neurons to generate CO_2 from glutamate reflects intrinsic cell properties rather than restrictions imposed by medium/cell-volume ratio, clustered or dispersed states, suspended or substratum-attached conditions, or time *in vitro*.

(b) The same ability to generate CO_2 from glutamate (in the presence of appropriate α -oxo acids) can ensure neuronal survival in 24 h cultures, and therefore must

meet energy or other metabolic needs of those neurons which glucose itself is unable to meet. However, one cannot yet assume that the inability of the neurons to satisfy their survival requirements with glucose is intrinsic to the initial dissociated cell preparation like their ability to utilize glutamate (concurrently with α -oxo acid), rather than acquired as time passes *in vitro*.

(c) The amount of $^{14}\text{CO}_2$ produced by neuronal suspensions from [^{14}C]glutamate and α -oxo acids under optimal substrate concentrations may represent the amount of energy-producing cell activity. Such a conclusion would be warranted if (i) CO_2 is not produced from other energy substrates in the same preparation (a plausible expectation, given the lack of survival in the absence of glutamate and/or α -oxo acid), and (ii) any $^{14}\text{CO}_2$ produced from [^{14}C]glutamate by decarboxylation or other non-energy-yielding reactions is either negligible (accessible by use of non-uniformly labelled glutamate), or constant and deductible (as is the case here).

Use of other selectively radioactively labelled substrates which support CNS neuronal survival (specifically pyruvate, oxaloacetate or oxoglutarate) will be useful in verifying that CO_2 -release measurements can evaluate (and even predict) neuronal survival regardless of the CO_2 sources. Such experiments may also provide information on metabolic-pathway involvements in the glucose inadequacy, and in particular where a restriction may occur in the conversion of exogenous glucose into (and use as) endogenous pyruvate.

The availability of a system in which CO_2 measurements can be matched to neuronal survival may be useful as a tool in examining the neuronal death phenomenon. A decline in $^{14}\text{CO}_2$ production may precede neuronal death long enough to permit an evaluation of reversible versus irreversible damage, e.g. by restoring trophic support after various periods of trophic deprivation. It may thus be possible to identify a short-latency response which will predict the long-term consequences of trophic deprivation in PNS neurons, much as has been done for Nerve Growth Factor and the Na^+/K^+ -pump in its target ganglionic neurons (Skaper & Varon, 1983).

No information is available at present as to why these embryonic CNS neurons fail to survive in a glucose-containing medium unless pyruvate or suitable α -amino acid/ α -oxo acid pairs are supplied to them. Since the requirement for the amino acid/oxo acid combinations (or for pyruvate) is considerably decreased by the sparing agents, an understanding of the mechanism of the sparing action may shed light on the uses by CNS neurons of these presumed energy sources. The ability of sparing agents to support maximal $^{14}\text{CO}_2$ production from such lower [^{14}C]glutamate concentrations implies that sparing agents increase the relative availability or needs of glutamate for CO_2 -releasing processes rather than, or in addition to, other glutamate utilizations.

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REFERENCES

- Barbin, M., Manthorpe, M. & Varon, S. (1984) *J. Neurochem.* **43**, 1468–1478
- Facci, L., Skaper, S. D. & Varon, S. (1985a) *J. Neurochem.* **45**, 926–934
- Facci, L., Skaper, S. D. & Varon, S. (1985b) *J. Neurosci. Res.*, in the press

- Manthorpe, M., Varon, S. & Adler, R. (1981) *J. Neurochem.* **37**, 759–767
- Manthorpe, M., Nieto-Sampedro, M., Skaper, S. D., Lewis, E. R., Barbin, G., Longo, F. M., Cotman, C. W. & Varon, S. (1983) *Brain Res.* **267**, 47–56
- McKeehan, W. L. & McKeehan, K. A. (1979) *J. Cell. Physiol.* **101**, 9–16
- Newman, R. E. & McCoy, T. A. (1958) *Proc. Soc. Exp. Biol. Med.* **98**, 303–306
- Selak, I., Skaper, S. D. & Varon, S. (1985) *J. Neurosci.* **5**, 23–28
- Sens, D. A., Hochstadt, B. & Amos, H. (1982) *J. Cell. Physiol.* **110**, 329–335
- Skaper, S. D. & Varon, S. (1983) *Brain Res.* **271**, 263–271
- Skaper, S. D., Molden, D. P. & Seegmiller, J. E. (1976) *Biochem. Genet.* **14**, 527–539
- Skaper, S. D., Adler, R. & Varon, S. (1979) *Dev. Neurosci.* **2**, 233–237
- Skaper, S. D., Selak, I., Manthorpe, M. & Varon, S. (1984) *Brain Res.* **302**, 281–290
- Varon, S., Nomura, J., Perez-Polo, J. R. & Shooter, E. M. (1972) in *Methods and Techniques of Neuroscience* (Fried, R., ed.), pp. 203–229, Marcel Dekker, New York
- Varon, S., Skaper, S. D., Barbin, G., Selak, I. & Manthorpe, M. (1984) *J. Neurosci.* **4**, 654–658

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