J. Physiol. (1971), **215**, *pp.* 103–117
With 7 text-figures
Printed in Great Britain

THE UPTAKE OF [^{14}C]GLYCINE BY SLICES OF MAMMALIAN SPINAL CORD

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(*Received 8 December 1970*)

SUMMARY

1. The accumulation of [^{14}C]glycine by slices of mammalian spinal cord has been measured.

2. When slices of rat cord were incubated at 37° C in a medium containing [^{14}C]glycine, tissue:medium ratios of about 30:1 were attained after a 40 min incubation.

3. After incubations at 37° C for 40 min, almost all (98%) the radioactivity in the tissue was present as unchanged [^{14}C]glycine.

4. The process responsible for [^{14}C]glycine uptake showed many of the properties of an active transport system: it was temperature sensitive, required the presence of sodium ions in the external medium, was inhibited by dinitrophenol and ouabain and showed saturation kinetics.

5. The estimated K_m value of glycine was $3{\cdot}1 \times 10^{-5}$ M, and V_{max} was 0·48 μ-mole/min.g cord.

6. The uptake of [^{14}C]glycine was not affected by the presence of large molar excesses of L-histidine, L-proline, L-aspartate, L-glutamate, L-valine or GABA, but was inhibited in the presence of L-alanine and L-leucine.

7. The uptake of [^{14}C]glycine was not reduced by strychnine, but a significant reduction in uptake was produced by *p*-hydroxymercuribenzoate.

8. The uptake of [^{14}C]glycine by the grey matter of rabbit spinal cord was 2 to 6 times greater than the uptake by slices of white matter incubated under the same conditions.

9. Rat cerebral cortex, cerebellar cortex and medulla also accumulated [^{14}C]glycine, and the uptake by the tissue slices *in vitro* appeared to parallel the concentration of glycine in these areas *in vivo*.

10. It is suggested that the glycine uptake system may represent a possible mechanism for the inactivation of glycine at inhibitory synapses in the spinal cord.

INTRODUCTION

Glycine occurs in the mammalian central nervous system in relatively large amounts (1–5 μmole.g) and is involved in many intermediary metabolic processes. In addition to this role in cerebral metabolism, it was suggested by Aprison & Werman (1965), on the basis of the distribution of glycine in the cat spinal cord, that this amino acid may have an additional action as an inhibitory synaptic transmitter in the cord, and there is now a considerable amount of neurochemical and neurophysiological evidence to support this suggestion (Davidoff, Shank, Graham, Aprison & Werman, 1967; Graham, Shank, Werman & Aprison, 1967; Werman, Davidoff & Aprison, 1967, 1968; Curtis, Hösli & Johnston, 1967, 1968; Curtis, Hösli, Johnston & Johnston, 1968; Ten Bruggencate & Engberg, 1968; Davidoff, Aprison & Werman, 1969).

However, although glycine now appears to fulfil many of the criteria for a neurotransmitter substance in the spinal cord, it is usually considered desirable to demonstrate the release of a putative transmitter from presynaptic terminals and to show that such a release is related to the frequency of stimulation of the appropriate nervous pathways.

It has proved difficult to demonstrate the neurally evoked release of glycine from the mammalian spinal cord *in vivo*, although the spontaneous release of [^{14}C]glycine, from superfused slices of rat spinal cord has been demonstrated, and this release was increased when the tissue was depolarized by electrical stimulation or by medium containing a high concentration of potassium chloride (40 mM) (Hopkin & Neal, 1970). Recently, Aprison (1970) has shown the release of [^{14}C]glycine from the superfused, toad spinal cord *in vitro* and has claimed that the amount of [^{14}C]glycine released was related to the frequency of stimulation of the dorsal roots.

The demonstration of transmitter release from cholinergic and adrenergic synapses was found to be greatly facilitated when the processes normally responsible for terminating the actions of the released transmitter substances were inhibited (Dale & Feldberg, 1934; Dale, Feldberg & Vogt, 1936; MacIntosh & Oborin, 1953; Brown, 1965). However, the mechanisms responsible for terminating the actions of the spinal inhibitory transmitter and iontophoretically applied glycine are unknown. Because the enzyme inhibitor *p*-hydroxymercuribenzoate was found to potentiate the inhibitory action of glycine on spinal interneurones (Curtis *et al.* 1968), it was suggested that glycine may be inactivated by enzymic degradation. However, mammalian brain tissue is capable of accumulating exogenous glycine (Smith, 1967) and so an alternative method of inactivation may involve an uptake mechanism in the neural tissue of the cord, similar to that described by Iversen & Neal (1968) for GABA in the cere-

bral cortex. This method of terminating the actions of these amino acids in the central nervous system would be analogous with the situation which exists at mammalian adrenergic synapses, where the actions of released noradrenaline are thought to be terminated by an uptake process (Iversen, 1967).

The present experiments were undertaken, therefore, to establish the properties of the glycine uptake system in mammalian spinal cord. Preliminary results of some of these studies have been reported previously (Neal, 1969; Neal & Pickles, 1969).

METHODS

Uptake of [^{14}C]glycine by slices of spinal cord

Male Wistar rats (100–140 g) were killed by a blow on the neck and the spinal cords were rapidly removed and chilled. The caudal section of each cord, consisting mainly of the lumbar enlargement, was weighed and placed on an iced surface. A slit was made along the dorsal surface of the cord and it was opened out to give a flat slab of tissue (50–70 mg). The cord was cut at 0·1 mm intervals with a mechanical chopper (McIlwain & Buddle, 1953) in two directions at 45°. The resulting slices (0·1 × 0·1 × approx. 1·0 mm) were suspended in 5 volumes of ice-cold incubation medium; dispersion of the tissue slices was facilitated by use of a Pasteur pipette. Portions of the tissue suspension containing the equivalent of 10 mg of cord were distributed volumetrically into a series of 25 ml. conical flasks containing 9·5 ml. ice-cold incubation medium. The flasks were gassed with a mixture of oxygen (95 %) and carbon dioxide (5 %) and fitted with rubber seals.

Slices were given a preliminary incubation for 15 min; then 0·5 ml. of incubation medium containing [^{14}C]glycine was injected through the rubber seal and the incubation was continued for various periods. Unless otherwise stated preliminary incubations and incubations were performed at 37° C which was found to be the optimal temperature for [^{14}C]glycine accumulation. The final concentration of [^{14}C]glycine in the incubation medium was $6{\cdot}0 \times 10^{-7}$ M in most experiments. At the end of the incubation, the tissue slices were recovered by rapid filtration on a small Buchner funnel fitted with a Whatman No. 1 filter paper disk (2·0 cm diameter); the filter was then rinsed with 5 ml. ice-cold incubation medium. The filter disks with the tissue slices were placed in scintillation vials and the [^{14}C]glycine was extracted by soaking the tissue for 60 min in 0·2 ml. distilled water. The radioactivity was measured by liquid scintillation counting after the addition of 4·0 ml. ethoxyethanol and 10 ml. phosphor (1 % Butyl P.B.D. Ciba, in toluene). Preliminary experiments established that there was no significant loss of [^{14}C]glycine from the slices during the washing procedure and that the wash removed most of the radioactivity in the incubation medium remaining on the filter paper. The results were corrected for the presence of the small amount of radioactivity which remained on the filter paper after washing (0·13 mμc).

In six experiments the spinal cord was removed from rabbits lightly anaesthetized with pentobarbitone sodium. The lumbar enlargement was placed on an iced surface and separated into grey and white matter. Slices were then prepared and incubated as described previously. Preliminary experiments in which the uptake of [^{14}C]-glycine by the cords obtained from anaesthetized rats and non-anaesthetized controls indicated that pentobarbitone sodium did not affect the accumulation of [^{14}C]glycine.

Efflux of [^{14}C]glycine from slices of rat spinal cord

Slices of cord were incubated with [^{14}C]glycine (6×10^{-7} M) for 40 min at 37° C and then recovered by filtration. After washing with 5 ml. of ice-cold medium, the filter paper, together with the slices, was placed in a flask containing 5 ml. of fresh medium at 37° C. The slices were incubated for various periods of time and then 0·2 ml. of the incubation medium was removed and the radioactivity released from the tissue was measured. On completion of the incubation, the radioactivity remaining in the slices of cord was extracted into the medium by the addition of Triton X–100 (10 μl.) and a further 0·2 ml. sample was removed to obtain an estimate of the total radioactivity remaining in the tissue at the end of the incubation.

Materials

The incubation medium was Krebs-bicarbonate Ringer of the following composition (g/l):

NaCl = 6·92, KCl = 0·354, $CaCl_2$ = 0·28, $MgSO_4$ = 0·144, KH_2PO_4 = 0·162, $NaHCO_3$ = 2·1, D-glucose 2·0.

Glycine-1-[^{14}C]. Specific activity 41·4 mc/m-mole was obtained from the Radiochemical Centre, Amersham.

RESULTS

Uptake and metabolism of [^{14}C]glycine

When slices of cord were incubated at 37° C in a medium containing [^{14}C]glycine (6×10^{-7} M) there was a rapid accumulation of radioactivity in the tissue resulting in a maximum tissue/medium ratio ([^{14}C]glycine, d.p.m./g tissue:[^{14}C]glycine, d.p.m./ml. medium) of 30:1 after a 40 min incubation (Fig. 1).

Because of the high tissue/medium ratios attained, it was important to maintain a large volume of incubation medium in relation to the amount of tissue (10 mg tissue in 10 ml. medium); under these conditions the concentration of [^{14}C]glycine in the incubation medium fell by less than 8% during a 60 min incubation. In three experiments in which slices of cord were incubated for 40 min, tissue extracts which contained 98% of the total radioactivity were concentrated and subjected to ascending paper chromatography, using butanol, acetic acid, water (8:5:3) as the solvent system. Only a single radioactive spot, corresponding in R_F to authentic glycine, was detected. The tissue protein remaining after the extraction procedure was dissolved in hyamine and the radioactivity was measured; less than 2% of the [^{14}C]glycine was incorporated into protein.

In subsequent experiments, therefore, the uptake of [^{14}C]glycine was measured by following the accumulation of total radioactivity in the tissue.

Time course of [^{14}C]glycine uptake

The time course of [^{14}C]glycine accumulation in slices of spinal cord incubated with [^{14}C]glycine (6×10^{-7} M) is illustrated in Fig. 1. There was an initial phase of very rapid uptake (0–10 min), followed by a somewhat slower linear phase (10–40 min). The maximum accumulation of [^{14}C]-glycine occurred after 40 min and a decline in the accumulation of [^{14}C]-glycine occurred when the tissue was incubated for a longer time (60 min).

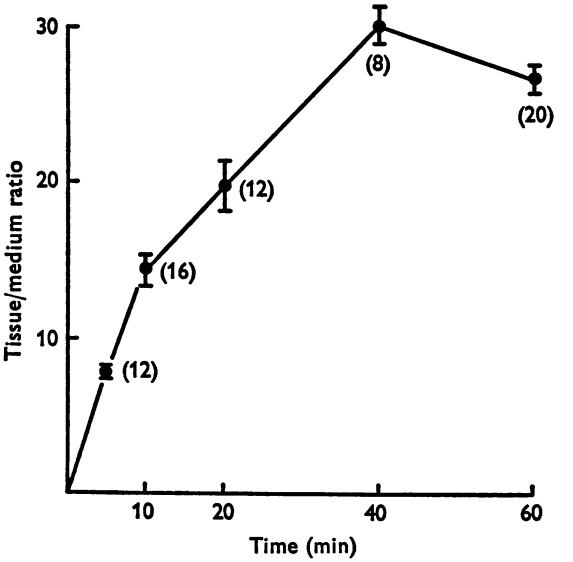

Fig. 1. Time course of [^{14}C]glycine uptake in slices of spinal cord incubated at 37° C with [^{14}C]glycine ($6{\cdot}0 \times 10^{-7}$ M). Figures in parentheses indicate number of experiments at each time; vertical bars indicate S.E. of mean.

Effect of temperature on [^{14}C]glycine uptake

When incubations were performed at 0 or 15° C the rate of uptake of [^{14}C]glycine was markedly reduced when compared with that at 37 or 25° C (Fig. 2). The values obtained at 15 and 25° C indicate a Q10 of approximately 2·0 for the uptake process in this temperature range. Although the rate of uptake was greatly reduced at 0° C, some uptake of [^{14}C]glycine still occurred at this temperature, giving a tissue:medium ratio of 2·6:1 after a 40 min incubation. Incubations at 37 and 25° C gave similar results but the accumulation of [^{14}C]glycine at 37° C was consistently greater than at 25° C, particularly during the initial rapid phase of uptake (0–10 min).

Effect of glycine concentration

The effects of glycine concentrations on the uptake of [^{14}C]glycine was examined by incubating slices of cord in media containing [^{14}C]glycine (6×10^{-7} M) and various amounts of non-radioactive glycine to give final concentrations ranging from 10^{-6} to 10^{-4} M. The values obtained after 10 min incubations were used to obtain approximate estimates of the initial rate of glycine uptake at the various glycine concentrations. These values

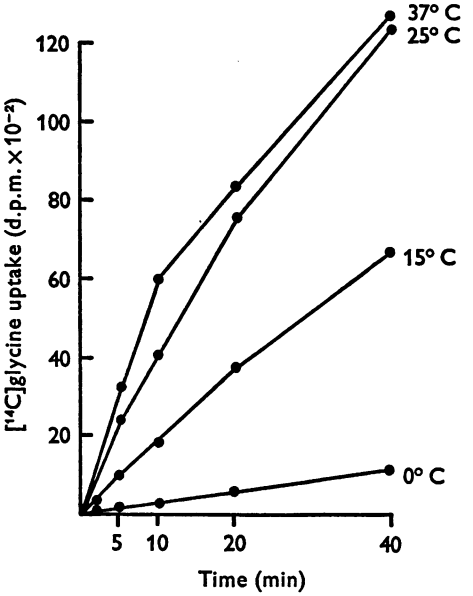

Fig. 2. Effect of temperature on [^{14}C]glycine uptake by slices of spinal cord incubated with [^{14}C]glycine ($6{\cdot}0 \times 10^{-7}$ M). Each point is the mean value for at least six experiments.

were found to lie on a straight line when plotted in a reciprocal form of the Michaelis–Menten equation (Fig. 3), indicating that glycine uptake is mediated by a saturable process. The apparent K_m for glycine uptake is $3{\cdot}1 \times 10^{-5}$ M and the V_{max} is 0·48 μmole/min.g fresh tissue 37° C.

Effect of sodium ion concentration on [^{14}C]glycine uptake

Slices of cord were incubated for 10 min in media in which Tris/hydrochloric acid buffer pH 7·4 (50 mM) was substituted for the normal bicarbonate buffer and in which varying proportions of the normal sodium chloride content were replaced by choline chloride or sucrose. The uptake of [^{14}C]glycine in sodium-free medium was reduced to zero. The results obtained from incubations in media containing various amounts of sodium are illustrated in Fig. 4.

Effects of metabolic inhibitors and drugs on [^{14}C]glycine uptake

Tissue slices were preincubated for 15 min in media containing the inhibitor or drug. [^{14}C]glycine and non-radioactive glycine (final concentration 10^{-5} M) were then added and the incubation was continued for

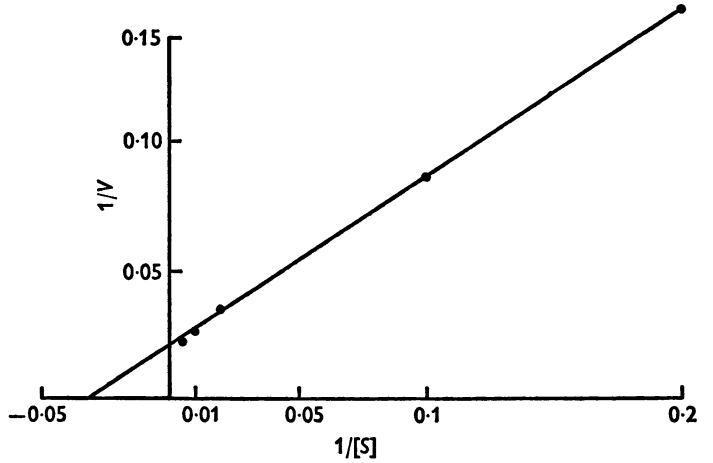

Fig. 3. Kinetic analysis of effect of glycine concentration on rate of [^{14}C]glycine uptake. V = rate of glycine uptake (10^{-8} mol/min.g cord), S = glycine concentration (10^{-6} M). Each value is the mean of six experiments.

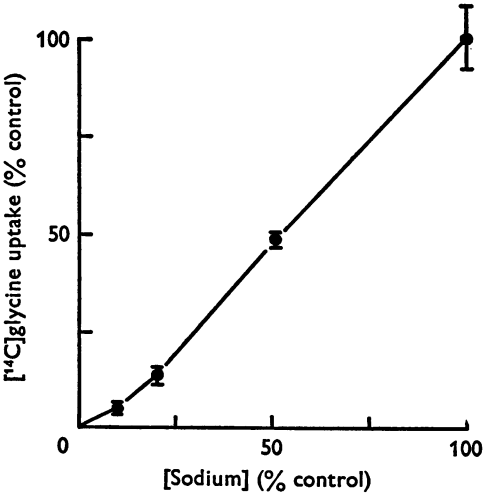

Fig. 4. Effect of sodium ion concentration on [^{14}C]glycine uptake in slices of spinal cord incubated for 10 min with [^{14}C]glycine ($6{\cdot}0 \times 10^{-7}$ M) in media in which various proportions of normal sodium content were replaced with Tris buffer and choline chloride. Each point is the mean ± S.E. of mean of four experiments.

10 min. The results are illustrated in Fig. 5. The presence of 2,4-dinitrophenol (10^{-3} M) or ouabain (10^{-5} M) caused a large reduction in [^{14}C]-glycine uptake. *p*-Hydroxymercuribenzoate at a concentration of 10^{-6} M did not affect the uptake of [^{14}C]glycine but when the concentration was increased to 10^{-5} M, the uptake of [^{14}C]glycine was reduced to 50% of the control values. Strychnine (10^{-3} M) did not significantly reduce the uptake of [^{14}C]glycine by the slices of cord.

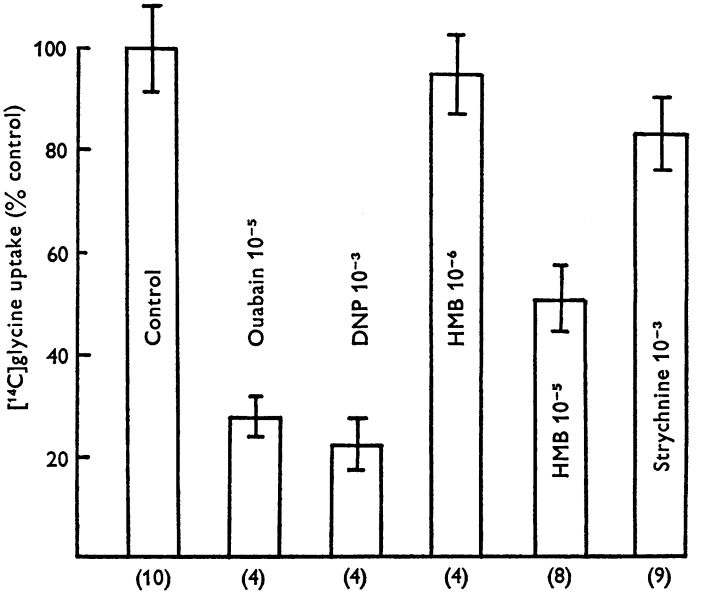

Fig. 5. Effect of pre-incubation and incubation with metabolic inhibitors or strychnine on [^{14}C]glycine uptake in slices of spinal cord incubated at 37° C with [^{14}C]glycine (10^{-5} M) for 10 min. Each value is the mean ± S.E. of mean of four experiments. DNP = 2,4-dinitrophenol; HMB = *p*-hydroxymercuribenzoate.

Effects of amino acids on [^{14}C]glycine uptake

Tissue slices were incubated for 10 min with [^{14}C]glycine (6×10^{-7} M) in the presence of various amino acids. L-Histidine, L-proline, L-valine, L-glutamate, L-aspartate and GABA had no effect on [^{14}C]glycine uptake at concentrations of 10^{-3} M. L-Alanine and L-leucine produced a significant inhibition of [^{14}C]glycine uptake at concentrations of 10^{-3} M. These results are summarized in Table 1.

Uptake of [^{14}C]glycine by grey and white matter from rabbit cord

Slices of grey matter from rabbit cord accumulated [^{14}C]glycine more rapidly than those from white matter; the maximum ratios of

tissue:medium which were attained after 30 min incubation were 16·3 and 2·9 respectively. The ratio of tissue:medium obtained with slices of whole cord fell between the values obtained for grey and white matter. The results are summarized in Table 2.

TABLE 1. Effects of amino acids on [^{14}C]glycine uptake

	[^{14}C]glycine uptake as % control
Control	100 ± 7·0
L-Histidine	79 ± 8·3
L-Leucine	75 ± 5·4*
L-Proline	82 ± 14·6
L-Alanine	76 ± 3·9*
L-Aspartate	102 ± 6·9
L-Glutamate	88 ± 4·9
L-Valine	91 ± 5·6
GABA	93 ± 7·7

[^{14}C]glycine uptake was measured in cord slices after a 10 min incubation at 37° C with [^{14}C]glycine (6×10^{-7} M) in the presence of various amino acids (10^{-3} M). Results are mean values ± S.E. of mean of four experiments.

* $P < 0{\cdot}05$ when compared with control.

TABLE 2. Uptake of [^{14}C]glycine by slices of grey matter and white matter from rabbit spinal cord

Time (min)	Tissue: medium ratio ± S.E. of mean		
	White matter	Grey matter	Whole cord
20	2·1 ± 0·18	4·5 ± 0·12	3·4 ± 0·22
30	2·9 ± 0·15	16·3 ± 2·10	5·0 ± 0·25
40	3·2 ± 0·20	12·8 ± 1·78	8·7 ± 0·53

[^{14}C]glycine uptake was measured in slices of grey matter, white matter, and whole cord after incubation at 37° C for various lengths of time. Results are the mean values ± S.E. of mean of six experiments.

Uptake of [^{14}C]glycine by different areas of rat brain

Rat brains were dissected and tissue slices were prepared from cerebral cortex, cerebellum, and medulla in addition to spinal cord. The slices from these different areas of brain were then incubated with [^{14}C]glycine (6×10^{-7} M) for 10 min at 37° C. The slices of tissue from all the areas studied accumulated [^{14}C]glycine and the uptake appeared to be related to the endogenous glycine levels. These results are illustrated in Fig. 6 where the uptake of [^{14}C]glycine has been plotted against the endogenous concentration of glycine. There was a significant correlation ($f = 0{\cdot}95$) between the glycine content of the tissue and its ability to accumulate [^{14}C]glycine.

Efflux of [^{14}C]glycine from slices of spinal cord

Slices of spinal cord were incubated with [^{14}C]glycine for 40 min at 37° C and then recovered by filtration. After washing, the slices were transferred to fresh medium and the incubations were continued for various periods in the absence of glycine.

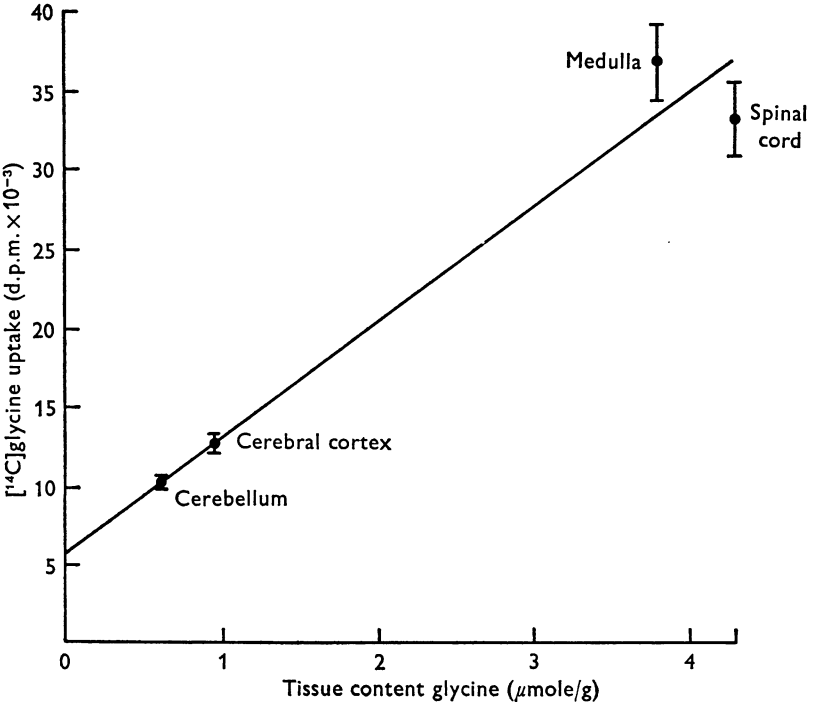

Fig. 6. Uptake of [^{14}C]glycine in slices of tissue prepared from different areas of rat brain and incubated for 10 min at 37° C with [^{14}C]glycine ($6{\cdot}0 \times 10^{-7}$ M). Each value is the mean ± S.E. of mean of five experiments. Apparently there is a significant correlation ($r = 0{\cdot}95$, $P < 0{\cdot}02$) between the uptake of [^{14}C]glycine and the tissue levels of glycine.

The efflux of [^{14}C]glycine from slices of cord was multiphasic and consisted of three major components (Fig. 7). An initial rapid efflux ($t_{\frac{1}{2}} \simeq 8$ min) was followed by a slower phase of release ($t_{\frac{1}{2}} \simeq 28$ min) and this was followed by a much slower efflux with a half-life in the order of 2 hr. Thus, after 30 min, 60% of the [^{14}C]glycine had been released into the external medium. However, in the following 30 min only a further 5% of the radioactivity was released, and at the end of a 60 min incubation the tissue retained 36% of the radioactivity taken up during the incubation period.

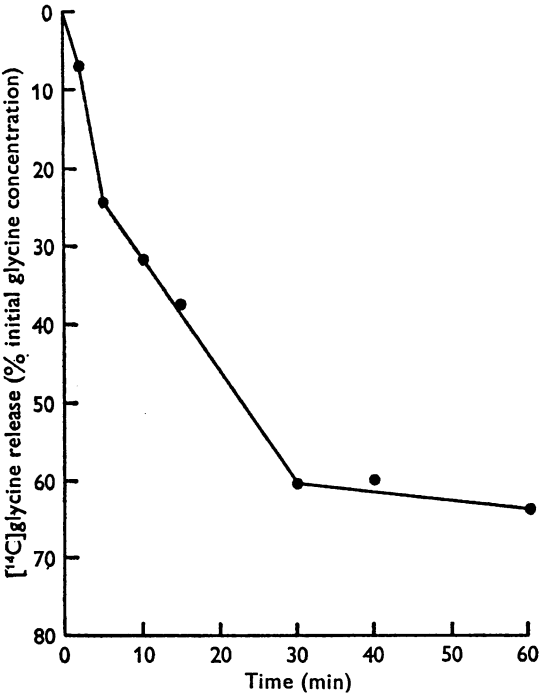

Fig. 7. Efflux of [^{14}C]glycine from spinal cord. Tissue slices were incubated for 40 min at 37° C with [^{14}C]glycine, recovered by filtration, and then placed in fresh medium. The release of [^{14}C]glycine from the slices into the medium is expressed as a percentage of [^{14}C]glycine taken up into the tissue during the 40 min incubation. Each value is the mean of four experiments.

DISCUSSION

The present results show that the mammalian spinal cord possesses the ability to concentrate glycine from an external medium, and also confirm several previous reports that other areas of the brain possess an uptake mechanism for this amino acid (Blasberg & Lajtha, 1965; Smith, 1967; Kandera, Levi & Lajtha, 1968; Barbosa, Joanny & Corriol, 1968).

Under the conditions used in the present experiments (low concentration of glycine, large volume of incubation medium compared with tissue, 37° C), slices of spinal cord show an appreciable ability to concentrate glycine, giving rise to tissue:medium ratios of about 30:1 after a 40 min incubation. Under these conditions, only a small fraction of [^{14}C]glycine is metabolized, 98% of the radioactivity in the tissue being present as [^{14}C]glycine. The process responsible for the accumulation of [^{14}C]glycine shows many of the properties characteristic of an active transport system:

thus, glycine is accumulated against a considerable concentration gradient, the uptake is temperature sensitive, sodium dependent, saturable and inhibited by dinitrophenol and ouabain.

Although a substantial efflux of [^{14}C]glycine from the cord was found to occur when tissue slices, which had been previously incubated with [^{14}C]glycine, were placed in fresh medium containing no glycine, it is unlikely that the uptake of [^{14}C]glycine is simply due to exchange diffusion with the large endogenous pool of glycine (4 μmole/g). The rapid phase of amino acid release from the slices occurs mainly during the 15 min preliminary incubation; also an exchange process would not be expected to exhibit the properties described above. Furthermore, it has been shown that slices of rat brain show a net increase in glycine when incubated in a medium containing this amino acid (Smith, 1967). It seems likely therefore that, in the present experiments, the accumulation of [^{14}C]glycine by slices of spinal cord represents an essentially unidirectional influx mediated by an active transport mechanism. The properties of this uptake mechanism are common to a number of other active transport systems, such as those responsible for sugar and amino acid transport in other tissues (Albers, 1967; Heinz, 1967).

The uptake of glycine by spinal cord is mediated by a process with considerable structural specificity, as it was found that the uptake of [^{14}C]-glycine was unaffected by the presence of large molar excesses of the amino acids: L-histidine, L-proline, L-aspartate, L-glutamate, L-valine and GABA. L-alanine and L-leucine produced small inhibitions of glycine uptake at a concentration of 10^{-3} M.

The precise cellular location of the uptake mechanism for glycine is unknown. It is not restricted to the neuronal cell bodies or nerve endings because slices of white matter from rabbit spinal cord were able to accumulate [^{14}C]glycine, although the uptake of this amino acid by white matter was 2 to 6 times less than the uptake by slices of grey matter incubated with [^{14}C]glycine under the same conditions. It has not yet been established whether the [^{14}C]glycine taken up by slices of cord mixes uniformly with the endogenous pools of glycine, nor have any studies on the subcellular distribution of endogenous glycine or [^{14}C]glycine in the cord been made. The multiphasic efflux of [^{14}C]glycine from slices of cord together with retention of a substantial proportion of the amino acid, suggests that [^{14}C]glycine is incorporated into several pools. The first phase of efflux, with a half-life of about 8 min, probably represents extracellular washout and the slowest phase, with a half-life in the order of 2 hr, is probably due to the release of [^{14}C]glycine from intracellular sites. The third component of the efflux curve, which had an intermediate half life of about 28 min, may represent the loss of radioactivity from cells damaged

by the slicing procedure. This is supported by results obtained from superfusion experiments using larger slices of spinal cord (chopped transversely at 1 mm intervals), where the efflux [^{14}C]glycine shows only two components with half lifes of approximately 10 min and 2 hr (J. M. Hopkin & M. J. Neal, unpublished observations). Similar biphasic effluxes of radioactive glycine, GABA and other amino acids from slices of cerebral cortex have been described (Srinivasan, Neal & Mitchell, 1969; Mitchell, Neal & Srinivasan, 1969).

Curtis *et al.* (1968) found that the enzyme inhibitor, *p*-hydroxymercuribenzoate potentiated the inhibitory action of glycine on spinal neurones and suggested that enzymic degradation may be involved in terminating the actions of glycine in the cord. However, the present results show that *p*-hydroxymercuribenzoate inhibits the uptake of glycine by the spinal cord and this action may be responsible for the potentiation of the effects due to glycine caused by the mercurial (Neal & Pickles, 1969; Neal, 1969). This suggestion is supported by the more recent work of Curtis, Duggan & Johnston (1970) who have found that *p*-hydroxymercuribenzoate potentiates the inhibitory actions on spinal neurones not only of glycine, but of GABA, β-alanine and α-alanine as well: Curtis *et al.* (1970) found that *p*-hydroxymercuribenzoate reduced the uptake of glycine, GABA, DL-aspartate and L-lysine by slices of cerebral cortex and suggested that uptake mechanisms in the cord may be involved in terminating the inhibitory actions of these amino acids. If glycine is a transmitter substance, and if it was inactivated by enzymic degradation, then it might be expected that its rate of synthesis would be faster in areas where glycine is thought to be a transmitter, i.e. spinal cord and medulla. However, Shank & Aprison (1970) found no apparent correlation between the rate of formation of glycine and the content in different areas. On the other hand, the present results indicate that the uptake mechanism for glycine does parallel the content in different areas and is most effective in the cord and medulla where glycine might be a neurotransmitter. Thus, the uptake mechanism for glycine in the cord may represent an effective mechanism for terminating its physiological actions after its release from inhibitory nerve endings.

It has been suggested that the amino acid uptake mechanisms in neural tissue may be concerned with regulating their endogenous levels in the brain because a number of amino acids show a parallelism between the level of the amino acid *in vivo* and its uptake by slices of brain *in vitro*. (Nakamura & Nagayama, 1966; Lajtha, 1967; Kandera, Levi & Lajtha, 1968; Battistin, Grynbaum & Lajtha, 1969). The present experiments in which the uptake of glycine was measured in four different areas of the rat central nervous system seem to confirm this parallelism between the

uptake of glycine *in vitro* and the tissue levels *in vivo*. Thus, the possibility exists that the uptake mechanism for glycine in the cord may be concerned with regulating the level of the endogenous glycine pool. Such a mechanism is not incompatible with the suggestion that the inhibitory synaptic actions of glycine might be terminated by uptake into the tissue, as such a process would also serve to maintain the tissue levels of this amino acid. A similar uptake mechanism may be responsible for terminating the inhibitory actions of GABA on cortical neurones (Iversen & Neal, 1968) and it is thought that an uptake mechanism is responsible for terminating the actions of noradrenaline after its release from adrenergic nerve terminals (Iversen, 1967). The presence of an efficient uptake mechanism for glycine in the spinal cord may well explain the difficulties encountered in demonstrating a neurally evoked release of this amino acid from the cord *in vivo*.

Part of this work was supported by the Medical Research Council. A grant for apparatus from the Smith Kline and French Foundation is also gratefully acknowledged.

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