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Chloride Dependence of the K^+-Stimulated Release of Taurine from Synaptosomes

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Exposure of a crude synaptosomal fraction to K^+ concentrations ranging from 25 to 100 mM evokes the release of [^{3}H]taurine and [^{3}H]GABA. These high concentrations of K^+ induce, besides depolarization, a marked synaptosomal swelling, which is prevented by replacing chloride in the solutions with the largely impermeant anion gluconate. The depolarizing effect of K^+ is unaffected by omission of chloride. The K^+-evoked release of taurine seems related to K^+-induced changes in synaptosomal volume rather than to a depolarizing effect, since it is totally calcium-independent but is abolished by reducing chloride and by making solutions hypertonic with mannitol. The release of [^{3}H]GABA, in contrast is unaffected in chloride-free or hypertonic solutions.

KEY WORDS: Taurine; synaptosome swelling; volume-sensitive release; chloride-dependent release.

INTRODUCTION

Taurine is released from a variety of nervous tissue preparations in response to depolarizing agents (1,2). This property of taurine has been often considered as supporting evidence for a neurotransmitter role of the amino acid. However, the features of taurine release evoked by depolarization show marked deviations from the release pattern of neurotransmitters occurring as consequence of the depolarization-secretion coupling of synapses. These differences include the calcium-independence of the release process and a delayed time course of the efflux (3–5). In the present study we observed another feature of taurine release which clearly differs from the normal behavior of neurotransmitters. This is the marked dependence of the K^+-evoked release on the presence of extracellular chloride. Experiments were designed to substantiate the interpretation that this chloride-dependent release of taurine is not consequence of K^+-induced depolarization but rather a response to swelling associated to high concentrations of K^+. With comparative purposes, the properties of GABA efflux were examined in parallel, under the same conditions as taurine release.

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EXPERIMENTAL PROCEDURE

Synaptosome Preparation and Superfusion. Wistar rats weighing about 200 g were used for obtention of brain cerebral cortex. The crude synaptosomal fraction was prepared by differential centrifugation according to Whittaker and Barker (6). Purified synaptosomes were obtained according to the procedure of Hajos (7). Synaptosome suspensions were preincubated under shaking with [^{3}H]taurine (4 μCi/ml) or [^{3}H]GABA (1 μCi/ml, 1μM final concentration) for 60 min and 20 mM, respectively in a Krebs-Ringer solution containing (in mM): NaCl 118; KCl 4.7, KH_2PO_4 1.17, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 10, adjusted to pH 7.4 by bubbling with O_2/CO_2 (95%: 5%). In some experiments, HEPES replaced bicarbonate as the buffer solution. After incubation, the synaptosomes were separated from preincubation solution by rapid filtration through a Millipore filter (pore size 0.65 μM) and rinsed with Krebs-Ringer medium. The filter membrane containing the synaptosomes was transferred to a glass superfusion chamber (0.25 ml volume) and superfused with normal medium bubbled with O_2/CO_2, at a flow rate of 0.6 ml/min. After a wash period

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of 12 min, fractions of the perfusate were collected at 1-min intervals directly into scintillation vials. The baseline was attained at about 6 min, after which the standard medium was replaced by medium containing different concentrations of KCl and the modifications or additions indicated at each experiment. High potassium media were made by substituting NaCl by equimolar amounts of KCl. In media with low chloride or in which the K × Cl product was constant, the isosmolarity and total anion concentration were maintained by replacement of chloride salts of sodium and potassium by the corresponding gluconate salts. In experiments to investigate the effect of omission of calcium or chloride, synaptosomes were exposed to modified media during all superfusion, i.e., including prestimulation and stimulation periods. For experiments with [^{3}H]GABA, incubation and superfusion media contained aminooxyacetic acid (0.1 mM). At the end of the superfusion, synaptosomes were solubilized and the radioactivity of collected fractions and that remaining in synaptosomes was measured by scintillation spectrometry. Results are expressed as the radioactivity released in fractions, in percent of total radioactivity accumulated by synaptosomes (radioactivity in samples plus radioactivity remaining in synaptosomes at the end of the superfusion).

Measurement of Membrane Potential. Membrane potential was monitored by tetraphenylphosphonium (TPP^+) according to Friedman et al. (8). Synaptosomes were incubated for 60 min at 37°C in the presence of [^{3}H]TPP^+ (0.1 μCi/ml) in the different experimental solutions. After incubation, the synaptosomal pellet was isolated by centrifugation, washed, solubilized and the radioactivity accumulated was measured by scintillation spectrometry.

Synaptosomal Volume. Volume of the synaptosome suspension was measured by a light-scattering technique according to Keen and White (9). The light scatter of synaptosome suspension was measured spectrophotometrically by recording changes in extinction at 700 nm. Synaptosomes were incubated with the different media in a thermostated cuvette equipped with a magnetic stirrer. Changes in extinction were followed during 10 min. Results are expressed in a relative scale, considering the change in synaptosomes incubated in Krebs-bicarbonate medium as 1.0. Experiments for volume measurements were carried out in a purified synaptosomal fraction. In most experiments, calcium was removed from the medium to avoid aggregation of synaptosomes and interference with the spectrophotometric determination. This modification did not significantly affect synaptosomal volume.

RESULTS

The efflux of [^{3}H]taurine from isolated nerve endings increased 2.5 fold by continuous exposure to 56 mM KCl. The time course of taurine release was slow in both onset and offset, the peak of stimulation being attained only 5 min after superfusion with KCl (Figure 1). The release of taurine increased with increasing concentrations of KCl. Stimulation with 25, 56, or 100 mM KCl resulted in an increased efflux of taurine of 1.5, 2.5, and 5 fold respectively over the spontaneous release (Figure 2).

Ionic Dependence of Taurine Release. The K^+-evoked release of taurine was totally calcium-independent. No inhibition of taurine release was observed in calcium-free media containing either 1 mM cobalt, 20 mM Mg or 200μM EGTA. Omission of calcium slightly increased the basal release, but the net stimulation release (i.e. stimulated minus basal release) was not significantly different from the release in the presence of calcium (Figure 1). Omission of chloride from the superfusion medium, replaced by the corresponding gluconate salts, totally suppressed the K^+-stimulated release of taurine at all K^+ concentrations (Figure 2). The spontaneous efflux was unaffected by omission of chloride (Figures 1, 2), but the inhibitory effect of chloride omission was higher and more clearly evidenced when synaptosomes were exposed to chloride-free medium through all the superfusion period. The release of taurine examined against a chloride-concentration curve, revealed that concentration of chloride required to elicit the release of taurine stimulated by K^+ was 64 mM (Figure 3). All these observations of taurine correspond to experiments using a crude synaptosomal fraction, but identical results were obtained in a purified synaptosomal preparation. The release of taurine evoked by K^+ (56 mM) decreased about 30% when HEPES replaced bicarbonate in the buffer solution in the Krebs-Ringer medium (not shown).

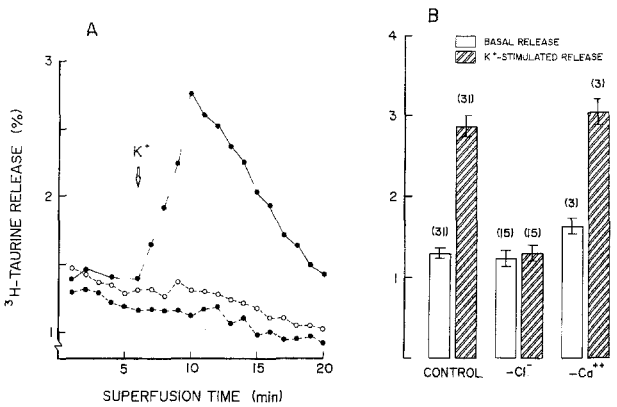

Fig. 1. Time-course and ion dependence of the K^+-stimulated release of [^{3}H]taurine from synaptosomes. Loading and superfusion were as described in Methods. Curves in A correspond to a representative experiment of [^{3}H]taurine release in a Krebs-bicarbonate medium stimulated at the arrow with 56 mM KCl (—●—), spontaneous [^{3}H]-taurine release (--●--) and stimulated release in a chloride free medium, (--○--). Bars in B represent the mean of the number of experiments indicated in parenthesis. White bars represent the spontaneous release and shaded bars the K^+-stimulated release in the presence or absence of Cl^- or Ca^{++}. The Cl^- free medium contained gluconate salts replacing NaCl and KCl. For the Ca^{++}-free conditions, $CaCl_2$ was omitted in the superfusion medium and replaced by $CoCl_2$ (1 mM). Spontaneous release corresponds to percent release of [^{3}H]taurine in the fraction prior stimulation (1 min) and stimulated release corresponds to the amount of [^{3}H]taurine released at the peak stimulation fraction. Results are the mean ± SEM.

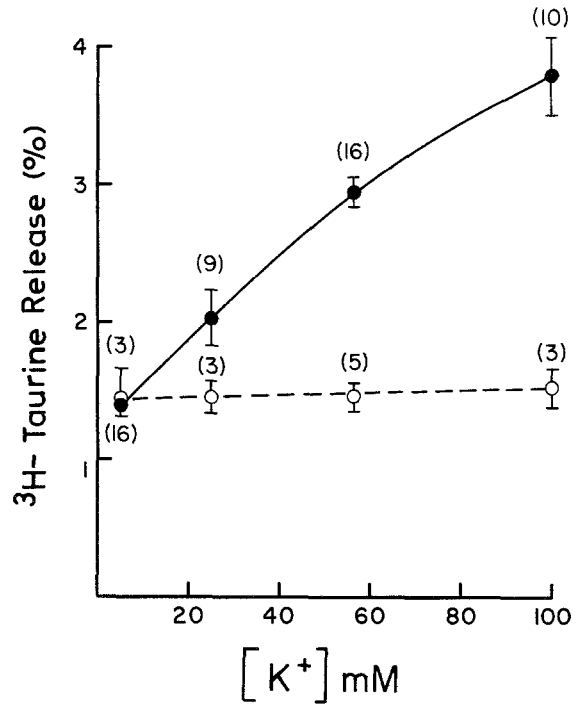

Fig. 2. Effects of increasing potassium concentration (•) and omission of chloride (○) on [^{3}H]taurine release from synaptosomes. Loading and superfusion were as described in Figure 1. Chloride-free solutions contained gluconate replacing chloride salts. Data in the curves represent the release (%) at the peak stimulation fraction during superfusion with the indicated concentrations of K^+. The point at 5 mM K^+ corresponds to the baseline release. Results are the mean ± SEM of the number of experiments indicated in parenthesis.

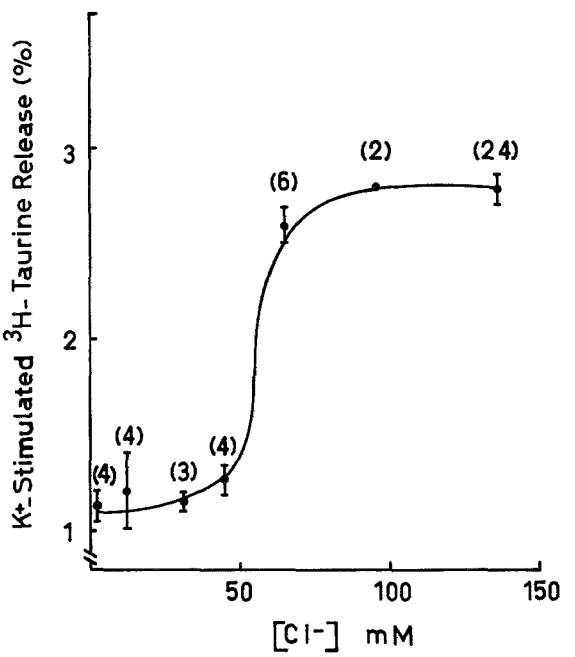

Fig. 3. Chloride-dependence of the K^+-stimulated release of [^{3}H]-taurine. Synaptosomes were superfused with media containing the indicated Cl^-- concentration from 2.5 to 125 mM and stimulated with 56 mM K^+. Chloride was replaced by gluconate. Results are the mean ± SEM of the number of experiments indicated in parenthesis.

The effect of chloride omission was examined on the K^+-stimulated release of [^{3}H]GABA. At a concentration of 56 mM, KCl enhanced the release of [^{3}H]-GABA from synaptosomes by about 8-fold. Omission of chloride had no effect on the K^+-evoked release of GABA but significantly increased the spontaneous release (Figure 4).

Effect of Chloride Omission on Swelling and Depolarization Associated to K^+. Significant swelling of the synaptosomal fraction occurred at 25 mM, 56 mM and 100 mM KCl concentrations. This effect was totally abolished when synaptosomes were incubated in a low chloride medium (Figure 5). Reduction in chloride concentrations did not inhibit the depolarizing effect of K^+ (Figure 6).

Effect of Anisosmotic Conditions in the K^+-Stimulated Release of Taurine. The effect of 56 mM K^+ was examined in solutions with normal concentrations of chloride but made hyperosmotic with mannitol (100 mM). The K^+-evoked release of taurine was abolished under these conditions whereas the release of GABA was unaffected (Figure 7). Incubation with hypoosmotic solutions (90% osmolarity) induced a volume increase of the synaptosomal fraction and a volume-sensitive [^{3}H]taurine release. This efflux was unaffected by omission of chloride.

Effect of Inhibitors of K^+-Fluxes. The effect of compounds which may inhibit K^+-accumulation by synaptosomes was examined on the K^+-evoked release of taurine. The inhibitors used were bumetanide, which blocks the K^+Cl^- cotransport, and barium, which antagonizes K^+ accumulation through calcium-dependent channels. None of these compounds modified the K^+-stimulated release of taurine.

Effect of Conditions Effecting the Taurine Transport System on K^+-Induced Efflux. In order to assess a possible involvement of the Na^+-dependent carrier transport for taurine on the K^+-stimulated release, the effect of sodium omission and the existence of taurine homoexchange, which would support a bidirectional movement of taurine were examined. Sodium omission did not affect the efflux of taurine, neither the spontaneous nor the K^+-stimulated release. Increasing the external concentration of taurine to 2 mM or 10 mM did not elicit

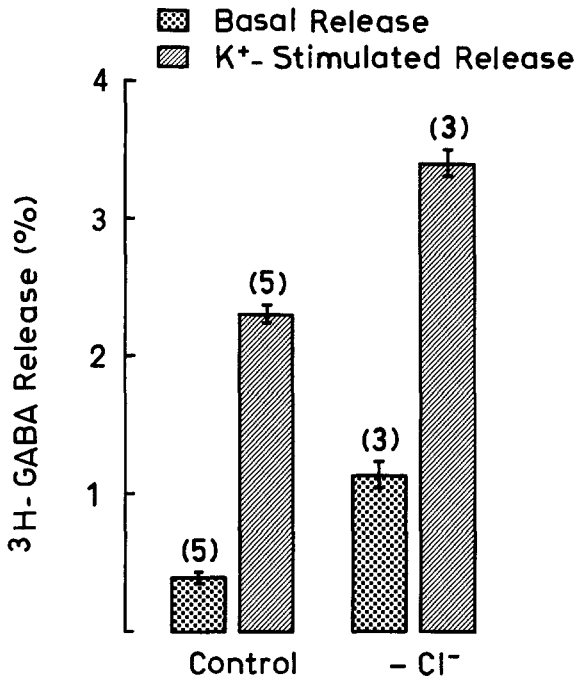

Fig. 4. Effect of chloride omission on the K^+-stimulated release of [^{3}H]GABA. Synaptosomes were superfused with a normal medium or with chloride-free medium and stimulated with 56 mM K^+. Chloride was replaced by gluconate. Results are the mean ± SEM of the number of experiments indicated in parenthesis.

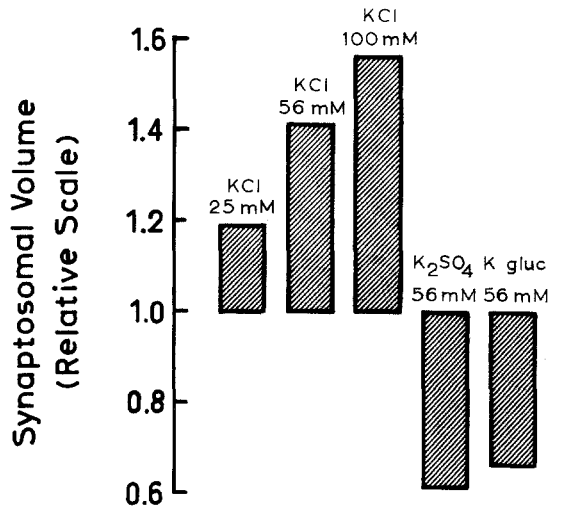

Fig. 5. Effects of increasing potassium concentrations and omission of chloride on synaptosomal volume. Swelling was measured by light scattering as described in Methods. The synaptosome volume is given relative to the change observed in synaptosomes incubated in Krebs-bicarbonate medium (5 mM KCl). Results are the mean of 6-8 experiments. SEM were lower than 12%.

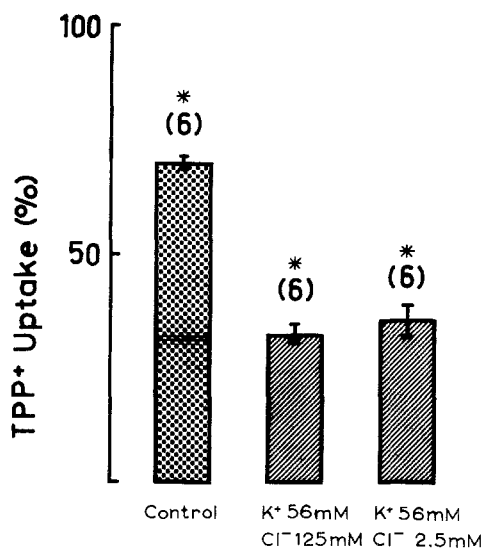

Fig. 6. Effect of 56 mm K^+ on the uptake of the [^{3}H]TPP^+ in the presence of normal or reduced chloride concentrations. Synaptosome suspension was incubated with [^{3}H]TPP^+ during 60 min. The low chloride media contained Na^+ and K^+ gluconate salts. Results are the mean ± SEM of the number of experiments indicated in parenthesis. *Significantly different by $P < 0.001$ (Student's t-test).

the release of previously accumulated [^{3}H]taurine, indicating the absence of taurine homoexchange (not shown).

DISCUSSION

The release of taurine from synaptosomes evoked by depolarizing agents, shows properties which do not conform with the neurotransmitter efflux pattern. Results of previous studies (3,10) and the observations of the present work, establish a number of differences in the K^+-stimulated release of taurine and that of neurotransmitters, namely: 1) a delayed time-course, slow in onset and offset; 2) the linearity of taurine efflux with K^+ concentrations; 3) the calcium-independence of the efflux process and 4) the chloride requirement of taurine release. All these observations indicate that the evoked release of taurine from synaptosomes is not consequence of an activation of the calcium mediated depolarization-secretion process typical of neurotransmitters.

In previous studies (11,12) the effect of chloride omission on the release of neurotransmitter amino acids has been examined in synaptosomes and the results showed that removal of chloride induces a marked increase of the spontaneous release of GABA and particularly of glutamate and aspartate, to an extent similar to the re-

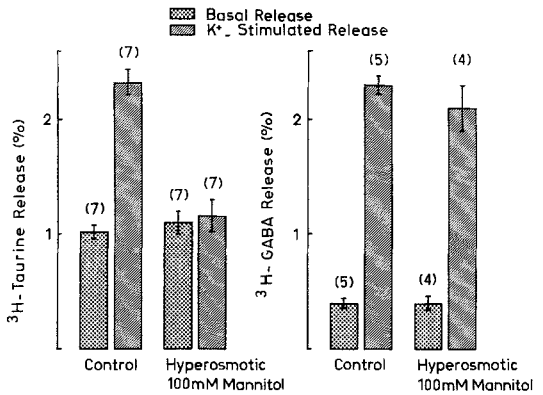

Fig. 7. Effect of a solution made hypertonic with mannitol on the K^+-stimulated release of [^{3}H]taurine and [^{3}H]GABA. Experimental conditions and expression of results are as described in Figure 1. Results are the mean ± SEM of the number of experiments indicated in parenthesis.

lease evoked by K^+. In the present study, omission of chloride increased the spontaneous efflux of GABA without modifying the stimulated release. An increased efflux of dopamine elicited by chloride removal has also been observed in chick retinas and rabbit striatal slices (13,14). The increased efflux of neurotransmitters induced by chloride omission has been considered as consequence of a depolarization occurring in the absence of chloride (15-17). In clear contrast with these results, the spontaneous release of taurine was not affected by removal of chloride but the K^+-evoked efflux was suppressed in these conditions, indicating that the release process is not linked to the depolarizing action of K^+. A chloride-dependence of K^+-stimulated release of taurine has been previously observed in chick and rat retina and in cultured astrocytes (13,18,19).

Increasing K^+ concentrations above 20 mM is known to induce swelling in a variety of nervous tissue preparations. Experiments in the present study were designed to investigate whether swelling associated to high K^+ concentrations is the signal for the chloride-dependent taurine release, since in all cases, replacement of chloride by an impermeant anion prevents the K^+-induced swelling. Bourke and coworkers reported marked swelling in mammalian cerebral cortex slices, proportional to external K^+ concentration. The K^+ induced swelling was increased when bicarbonate was used as buffer in the solution and was abolished by replacing chloride by the impermeant anion glucuronate (20-22). Similarly, Lipton (23) described that increases in K^+, even as low as 5 mM cause significant raises in cell volume in guinea pig cerebral cortex slices, and that marked swelling occurs with higher concentrations of K^+. In that study it was also shown that the K^+-induced increase in cell volume is abolished when Cl^- is replaced by glucuronate. The main cell type affected by swelling in brain slices is glia. However the effect of K^+ on inducing swelling is also observed in isolated nerve endings. Using a light-scattering technique, Kamino et al. (24) showed that nerve endings markedly swell when the external K^+ concentration exceeds 30 mM, an observation confirmed in this study.

Swelling associated to K^+ in nervous tissue seems to occur as consequence of K^+ accumulation mediated either by carrier transport systems or by passive diffusion governed by Donnan forces (25). Chloride and osmotically obliged water follow K^+into cells resulting in increased cell volume. In the conditions of the present study, decreasing Cl^- concentration only to an extent necessary to maintain the $K \times Cl$ product constant, abolished taurine release, suggesting that K^+ accumulation mediated by passive diffusion forces is the only process inducing the release of taurine.

Evidence from this work supports the interpretation that the K^+-stimulated taurine release is a response to swelling rather than to depolarization. The physiological significance of this volume-sensitive release of taurine from nerve endings is unclear at present. Neuronal structures change their volume under different physiological and pathological situations including spreading depression or hyperexcitability (26-28). It is possible that volume adjustment is required for the normal functioning of synaptic terminals as result of the excitotoxic release and subsequent uptake of neurotransmitters. A compound like taurine, which due to its inertness may be is mobilized across the membrane without affecting the cell metabolism, represents an ideal osmolyte. Taurine has a generalized, mild depressant action on neuronal activity (1,2), but this effect is observed at relatively high concentrations, which are unlikely to be attained in physiological conditions. However, when as consequence of hyperexcitability, nervous tissue swelling occurs, the volume-dependent release of taurine may participate not only in cell volume regulatory processes but also contribute to modulation of nervous excitability.

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REFERENCES

1. Oja, S. S, and Kontro, P. 1983. Taurine. Pages 501–533, A. Lajtha. Handbook of Neurochemistry, vol. 3, 2nd. ed. Plenum Press: New York.
2. Huxtable, R. J. 1989. Taurine in the central nervous system and the mammalian actions of taurine. Progr. Neurobiol. 32:472–533.
3. Placheta, P., Singer, E., Sieghart, W. and Karobath, M. 1979. Properties of ^{3}H-taurine release from crude synaptosomal fractions of rat cerebral cortex. Neurochem. Res. 4:703–711.
4. Hanretta, A. T. and Lombardini, J. B. 1987. Is taurine a hypothalamic neurotransmitter?: a model of the differential uptake and compartmentalization of taurine by neuronal and glial cell particles from the rat hypothalamus. Brain Res. 12:167–201.
5. Oja, S. S. and Kontro, P. 1987. Cation effects on taurine release from brain slices: Comparison to GABA. J. Neurosci. Res. 17:302–311.
6. Whittaker, V. P. and Barker, L. A. 1972. The subcellular fractionation of brain tissue with special reference to the preparation of synaptosomes and their component organelles. Pages 2-52, *in* Fried R. (ed.) Methods of Neurochemistry, Marcel Dekker, New York.
7. Hajós, F. 1975. An improved method for the preparation of synaptosomal fractions in high purity. Brain Res. 93:485–489.
8. Friedman, J. E., Lelkes, P. J., Lavie, Rosenheck, K., Schneeweiss, F., and Schneider, A. S. 1985. Membrane potential and catecholamine secretion by bovine adrenal chromaffin cells: use of tetraphenylphosphonium distribution and carbocyanine dye fluorescence. J. Neurochem. 44:1391–1402.
9. Keen, P., and White, T. D. 1970. A light-scattering technique for the study of the permeability of rat brain synaptosomes in vitro. J. Neurochem. 14:565–571.
10. Kontro, P. 1979. Components of taurine efflux in rat brain synaptosomes. Neuroscience. 4:1745–1749.
11. Hardy, J. A., Boakes, R. J., Thomas, D. J. E. Kindd, A. M., Edwardson, J. A., Virmani, M., Turner, J., and Dodd, P. R. 1984. Release of aspartate and glutamate caused by chloride reduction in synaptosomal incubation media. J. Neurochem. 42:875–877.
12. Naalsund, L. U., and Fonnum, F. 1986. Differences in anionic dependence of the synaptic efflux of D-aspartic acid and aminobutyric acid. J. Neurochem. 47:687–690.
13. Pasantes-Morales, H., Dominguez, L., Montenegro, J., and Morán, J. 1988. A chloride-dependent component of the release of labeled GABA and taurine from the chick retina. Brain Res. 459:120–130.
14. Diliberto, P. A., Jeffs, R. A., and Cubeddu, L. X. 1989. Effects of low extracellular chloride on dopamine release and the dopamine transporter. J. Pharm. Exp. Ther. 248:644–653.
15. Hodkin, A. L. and Horowicz, P. 1960. Potassium contractions in single muscle fibres. J. Physiol. (Lond.) 153:386–403.
16. Nishiyama, A., and Petersen, O. L. 1975. Pancreatic acinar cells: ionic dependence of acetylcholine-induced membrane potential and resistance change. J. Physiol. (Lond.) 224:431–465.
17. Turner, J. D., Boakes, R. J., Hardy, J. A., and Virmani, M. A. 1987. Efflux of putative transmitters from superfused rat brain slices induced by low chloride ion concentrations. J. Neurochem. 48:1060–1068.
18. Domínguez, L., Montenegro, J., and Pasantes-Morales, H. 1989. A volume-dependent, chloride-sensitive component of taurine release stimulated by potassium from retina. J. Neurosci. Res. 22:356–361.
19. Pasantes-Morales, H., and Schousboe, A. 1989. Release of taurine from astrocytes during potassium-evoked swelling. Glia 2:45–50.
20. Bourke, R. S. 1969. Studies of the development and subsequent reduction of swelling of mammalian cerebral cortex under isosmotic conditions *in vitro*. Exp. Brain Res. 8:232–248.
21. Bourke, R. S., and Tower, D. B. 1966. Fluid compartmentation and electrolytes of cat cerebral cortex *in vitro* I. Swelling and solute distribution in mature cerebral cortex. J. Neurochem. 13:1071–1097.
22. Bourke, R. S., and Kimelberg, H. K. 1975. The effect of HCO_3 on the swelling and ion uptake of monkey cerebral cortex under conditions of raised extracellular potassium. J. Neurochem. 25:323–328.
23. Lipton, P. 1973. Effects of membrane depolarization on light scattering by cerebral cortical slices. J. Physiol. (Lond.) 231:365–383.
24. Kamino, K., Inouye, K., and Inouye, A. 1973. Potassium ion-induced swelling of nerve-ending particles by light-scattering measurement. Biochim. Biophys. Acta 330:39–52.
25. Walz, W. 1986. Swelling and potassium uptake in cultured astrocytes. Can. J. Physiol. Pharmacol. 65:1051–1057.
26. Van Harreveld, A. and Khattab, F. 1967. Changes in cortical extracellular space during spreading depression investigated with the electron microscope. J. Neurophysiol. 30:911–929.
27. Trubatch, J., Loud, A., and Van Harreveld. 1977. Quantitative stereological evaluation of KCl-induced ultrastructural changes in frog brain. Neuroscience 2:963–974.
28. Soderfeldt, B., Kalimo, H., Olsson, Y., and Siesjo, B. 1981. Pathogenesis of brain lesions caused by experimental epilepsy. Light and electron microscopic changes in the rat cerebral cortex following bicuculline-induced status epilepticus. Acta Neuropathol. 54:219–321.