Biochemical Pharmacology, Vol. 34, No. 8, pp. 1217–1224, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

RELEASE OF D-[^{3}H]ASPARTIC ACID FROM THE RAT STRIATUM

EFFECT OF VERATRIDINE-EVOKED DEPOLARIZATION, FRONTO-PARIETAL CORTEX ABLATION, AND STRIATAL LESIONS WITH KAINIC ACID

LUIS ARQUEROS, JORGE ABARCA and GONZALO BUSTOS*
Laboratory of Biochemical Pharmacology, Department of Cell Biology, Pontificia Universidad Católica de Chile, Santiago, Chile

(*Received* 10 *January* 1984; *accepted* 31 *July* 1984)

Abstract—The spontaneous and depolarization-evoked release of radiolabeled D-aspartic acid, previously taken up by rat striatal slices, was studied by using a superfusion system. Veratridine (10–50 μM), electrical field stimulation (20 Hz, 1.0 V, 60 sec), and potassium (53 mM) markedly potentiated the release of D-[^{3}H]aspartate from striatal slices. The release of L-[^{3}H]glutamate was also increased by veratridine, according to a pattern and time course of release similar to that of D-[^{3}H]aspartate. However, the ratio of D-[^{3}H]aspartic acid release evoked by veratridine over spontaneous levels of release was much higher when compared to that of radiolabeled L-glutamate. Omission of calcium from the superfusion medium almost completely suppressed D-[^{3}H]aspartate release evoked by veratridine or by electrical stimulation whereas high K^+-evoked release of the [^{3}H]amino acid was only slightly reduced. However, increasing Mg^{2+} concentration to 12 mM in the superfusion medium did substantially block D-[^{3}H]aspartate release induced by K^+-depolarization. Additional experiments showed that tetrodotoxin (1 μM), a blocker of voltage-dependent Na^+ channels, totally abolished veratridine-evoked release of D-[^{3}H]aspartate from striatal slices. Finally, lesion studies showed that unilateral ablation of the fronto-parietal cortex was accompanied by a significant decrease in the high-affinity uptake of striatal D-[^{3}H] aspartate and by a large and parallel loss from striatal slices in D-[^{3}H]aspartate release evoked by either veratridine or high K^+. In contrast, unilateral injection of kainic acid into the striatum did not influence depolarization-evoked release of D-[^{3}H]aspartate from striatal slices. The findings reported suggest that D-[^{3}H]aspartic acid may be taken up preferentially and then released, in a Ca^{2+}-dependent manner, by veratridine and electrical stimulation from nerve terminals belonging to the cortico-striatal pathway. In addition, the results provide further support for the view that excitatory amino acids may act as neurotransmitters at the cortico-striatal nerve fibers.

There is increasing evidence which suggests that amino acids, such as L-glutamate and L-aspartate, may act as excitatory neurotransmitters within well defined neuronal pathways in basal ganglia structures [1, 2]. In particular, such a role for L-glutamate has been suggested for neuronal afferents, located in the striatum, that originate from various areas of the cerebral cortex [2, 3]. In support of this view, biochemical studies have shown that L-glutamic acid may be taken into striatal nerve terminals by a high affinity process and that destruction of cortico-striatal fibers are accompanied by a selective reduction of striatal glutamate levels as well as by a specific decrease in the high-affinity uptake of L-glutamic acid [4–6].

Critical evidence supporting L-glutamate or L-aspartate as putative transmitters of the cortico-striatal tract would be the demonstration of a specific release of these amino acids with the characteristics of a stimulus–secretion coupling, i.e. calcium dependence and a rapid rate of onset [2]. In fact, previous studies have shown that K^+-depolarization evoked the release, from striatal slices, of [^{3}H]glutamate newly synthesized from [^{3}H]glutamine [7, 8]. In a more recent study, release of endogenous L-glutamate from striatal slices was found to occur following the introduction of calcium ions during K^+-depolarization [9]. This type of experiment, however, may be criticized on two grounds. First, only high extracellular K^+ concentrations have been used as stimuli to produce depolarization. Depolarization with high K^+ may evoke the release of transmitter amino acids from nerve endings even in the absence of extracellular Ca^{2+} [10–14]. In fact, the rather poor Ca^{2+} dependence of K^+-depolarization-induced release of L-glutamate may indicate that this amino acid is not released from nerve endings but, rather, from glial structures [14, 15]. Second, the increase in L-glutamic acid release induced by elevated potassium over the prestimulation period is rather small, being only two or three times control, and probably reflects the widespread distribution of this amino acid in the nerve terminals, occurring not only in functional compartments related to release but also in compartments related to metabolic functions [2].

It is rather surprising that rigorous experimental

* Author to whom all correspondence should be addressed.

criteria have never been used when the release of excitatory amino acids from cortico-striatal projections has been studied. These experimental criteria have, in fact, been applied for other brain regions such as cerebral cortex, in which cortical slices, electrical field stimulation, tetrodotoxin (TTX), Ca^{2+} deprivation, and high Mg^{2+} have been used to identify the release of radiolabeled exogenous or endogenous L-glutamate or L-aspartate from nerve terminals [16, 17]. It is quite apparent that further information is needed, in regard to L-glutamate or L-aspartate release from cortico-striatal fibers, with agents or experimental conditions capable of evoking a depolarization that might be considered more physiological than that evoked with high extracellular K^+ concentrations. Both electrical field stimulation and micromolar concentrations of veratridine induce a type of depolarization mediated by cationic fluxes which resemble the fluxes that occur during physiological depolarization [18–20] and which, in addition, are abolished by TTX, a blocker of voltage-dependent Na^+ channels [19, 20].

It is apparent that experiments are also needed using structural analogues of L-glutamate and L-aspartate that do not enter the metabolic pool at the nerve terminal and which might provide information in regard to the roles these amino acids may play as putative transmitters of the cortico-striatal pathway. A number of studies indicate that D-[^{3}H]aspartic acid, a non-metabolized amino acid analogue, is taken up into striatal nerve terminals by a high-affinity process that does not differentiate between L-glutamate, L-aspartate and D-asparate [21–23]. In addition, the effect of destruction of cortico-striatal fibers is a decrease in the high-affinity uptake of both D-[^{3}H]aspartate and [^{3}H]glutamate [5, 6, 24]. These results suggest that D-[^{3}H]aspartate may be an excellent chemical marker of cortico-striatal nerve terminals. In fact, D-[^{3}H]aspartate has been used as a chemical marker in other central neurons that presumably use L-glutamate or L-aspartate as their transmitters, such as afferent fibers to hippocampus pyramidal cells [25], fibers from fimbria to dorsolateral septum [26], retino-tectal pathway [27], auditory system and cerebellar climbing fibers [28, 29].

It was therefore considered of interest to study in rat striatal slices the characteristics of D-[^{3}H]aspartic acid release following depolarizing stimuli such as those induced by veratridine or electrical field stimulation. The effects of fronto-parietal cortex lesions and intrastriatal kainic acid injection upon D-[^{3}H] aspartate release was also investigated in order to assess whether the induced-release of this amino acid is dependent on the presence of a specific neuronal pathway, namely the cortico-striatal afferents. Preliminary results from this study have been communicated elsewhere [30,*]. While this manuscript was in preparation, it was reported that electrical stimulation of striatal slices evokes a Ca^{2+}-dependent release of D-[^{3}H]aspartate [31].

* L. Arqueros, J. Abarca and G. Bustos, Abstr., Ninth Latinoamerican Congress of Pharmacology and Therapeutics, Oct. 17–23, Santiago, p. 88 (1982).

MATERIALS AND METHODS

Fronto-parietal cortex ablation. Male Sprague–Dawley rats (180–220 g) were anesthetized by intraperitoneal injections of pentobarbital (35 mg/kg). The skull overlying the right frontal and parietal cortex was removed with a dental drill. For fronto-parietal ablation the cortex was extensively lesioned by undercutting the cortex as described by McGeer *et al.* [5]. First, a transverse section of the right hemisphere was made 1.0 mm anterior to the caudate-putamen and a sagittal cut was made lateral to the caudate-putamen, 4.0 mm from the midline. Finally, the cortex between the frontal pole and the parietal-occipital suture was removed down to the white matter by suction through a fine hypodermic needle. The lesioned area was gently packed with Sterispon (Ferransan, Denmark), and the scalp was sutured. The animals were allowed to recover for 3 weeks prior to being killed. During later sectioning of the brain the extent of the lesion was also inspected. In no case did we observe that the lesion penetrated into the striatum. In sham-operated animals, the skull was opened, but no lesion made.

Striatal lesions with kainic acid. After anesthesia with pentobarbital (35 mg/kg, i.p.), the rats were positioned in a Narishige small-animal stereotaxic apparatus, and a Hamilton microliter syringe was inserted into the striatum through a burr hole in the calvarium. Stereotaxic coordinates, adapted from Schwarcz and Coyle [32], and based on the König and Klippel stereotaxic atlas [33], were: A 7.9, L 2.2, and V 4.0 mm. Kainic acid [2 μg in 1 μl Krebs–Ringer phosphate (KRP), pH 7.4] was injected at the rate of 0.2 μl/min into the left striatum, and the needle was left in for a further 5 min; the needle was then carefully removed and the scalp was apposed with sutures. Eight days after treatment the rats were decapitated, and striatal tissue slices were prepared to perform release studies as described below. The extent of the striatal lesion induced by kainic acid was determined by measuring choline acetyltransferase activity, a marker of intrinsic striatal neurons, according to the method of Schrier and Shuster [34] as modified by Arqueros and Abarca [35]. The specificity of the lesion was also determined through spectrofluorometric analysis of striatal dopamine content [36]. In all cases, the choline acetyltransferase activities and dopamine content in the contralateral uninjected striatum did not differ from controls; hence, in all the experiments the contralateral striatum was used as a control. The position of the lesion was checked in the first few animals with an injection of methylene blue to visualize the needle track.

Preparation and incubation of rat striatal slices. Each rat was decapitated, the brain was quickly removed, and the striatum was dissected at 4° as described by Glowinski and Iversen [37]. Striatal slices (2 × 2 × 0.2 mm) were prepared using a Sorvall tissue chopper. The slices (about 6–8 mg) were incubated for 5 or 30 min at 37° in 2 ml KRP at pH 7.4 saturated with 95% O_2–5% CO_2 and containing either L-[2,3-^{3}H]glutamic acid (sp. act. 18.8 Ci/mmole, final concentration 2.7×10^{-7} M) or D-[2,3-^{3}H]aspartic acid (sp. act. 13.8 Ci/mmole, final concentration 3.6×10^{-7} M). At the end of the incu-

bation period, the slices were rapidly separated from the medium through a 5.0 ml lucite chamber with a nylon mesh bottom.

D-[^{3}H]*Aspartic acid and* L-[^{3}H]*glutamic acid uptake.* The slices, incubated for 5 min as above with labeled D-aspartic acid or L-glutamic acid, were collected by rapid filtration, washed two times with 5 ml KRP solution, homogenized in trichloroacetic acid (15%, w/v), and centrifuged for 10 min at 12,000 *g*. The clear supernatant fractions were analyzed for tritium in a Nuclear Chicago Scintillation counter. Protein was measured according to the method of Lowry *et al.* [38] using bovine serum albumin as a standard.

D-[^{3}H]*Aspartic acid and* L-[^{3}H]*glutamic acid release from striatal slices.* The chemically-induced release of exogenously taken up ^{3}H-amino acid was followed essentially as described by Bustos and Roth [39]. Briefly, striatal slices, previously incubated for 30 min as described above with D-[^{3}H]aspartate or L-[^{3}H]glutamate, were transferred to superfusion chambers, washed with 10 ml KRP, and then superfused with KRP solution that was being continuously oxygenated and prewarmed to 37°. A constant flow rate of 4 ml/min was maintained by means of a peristaltic pump, and a two-way system was set up to switch to different superfusion solutions without disrupting the flow. An initial superfusion period of 20 min was allowed before chemically-induced release. Two samples of 1 min each were collected to determine the spontaneous release of the labeled amino acid. Stimulated release from striatal slices was induced by changing the superfusion solution for 1 min to KRP containing either veratridine (5–50 μM) or K^+ (53 mM). Thereafter, the superfusion fluid was changed back to normal KRP, and four additional samples of 1 min each were collected. The slices were then superfused for a further 10-min period with KRP solution before the next stimulation was induced. At the end of the experiment, the slices were recovered and the remaining radioactivity was extracted from the tissue with trichloroacetic acid (15%, w/v) and assayed for radioactivity by liquid scintillation counting.

The following procedure was used when electrically-induced release from striatal slices of exogenously loaded ^{3}H-amino acids was studied. The slices, previously incubated in the presence of labeled D-aspartic acid as described above, were transferred to superfusion chambers (2.0 ml capacity) with two circular platinum electrodes (distance between electrodes = 2.5 cm) to allow for electrical field stimulation [40]. The chambers permitted a continuous superfusion of the slices, at a constant flow rate of 4 ml/min, while stimulation was taking place. Once steady and constant basal levels of release were established, stimulation of release was evoked electrically. Electrical stimuli were applied for 1 min by means of Grass model S4 stimulator. The stimuli were applied in biphasic pulses of 5 msec duration each, at a frequency of 20 Hz and 1.0 V.

Calculation of release data. Stimulation-induced release of radioactivity in excess of baseline efflux was calculated and expressed as the percentage of total radioactivity present in the tissue at the onset of stimulation. Spontaneous release corresponded to the percentage of ^{3}H-amino acid released 1 min before the stimulation period and once steady basal levels of release were established. Results are expressed as mean ± S.E.M. unless stated differently; a Student's *t*-test was used for comparison of mean values.

Solutions and chemicals. The Krebs–Ringer–phosphate used in these experiments had the following composition: 128 mM NaCl, 4.8 mM KCl, 0.75 mM $CaCl_2$, 1.2 mM $MgSO_4$, 16 mM glucose and 16 mM sodium phosphate buffer (pH 7.4). Krebs–Ringer–phosphate–high K^+ was made by replacing proportion of NaCl with equimolar amounts of KCl. Other modifications of the KRP are described in the text. D-[2,3-^{3}H]Aspartic acid (13.8 Ci/mmole) and L-[3,4-^{3}H]glutamic acid (18.8 Ci/mmole) were purchased from the New England Nuclear Corp. Boston, MA, U.S.A. Veratridine was obtained from Merck, Darmstadt, FRG. Unlabeled amino acids and kainic acid were obtained commercially from the Sigma Chemical Co., St. Louis, MO, U.S.A.

RESULTS

Effects of veratridine on L-[^{3}H]*glutamic and* D-[^{3}H]*aspartic acid release from striatal slices.* Incubation of striatal slices for 30 min at 37° in the presence of 10 μCi of the labeled amino acids resulted in a substantial accumulation in the slices of the radioactivity (see legends of Figs. 1 and 2). Although each of the amino acids was used at similar final concentrations (approx. 3.5×10^{-7} M), the extent of the label that accumulated differed substantially depending on which ^{3}H-amino acid was studied. D-[^{3}H]Aspartic acid accumulation by striatal slices amounted to 67% of the total radioactivity used during the incubation, whereas L-[^{3}H]glutamic acid accumulation amounted only to 32%.

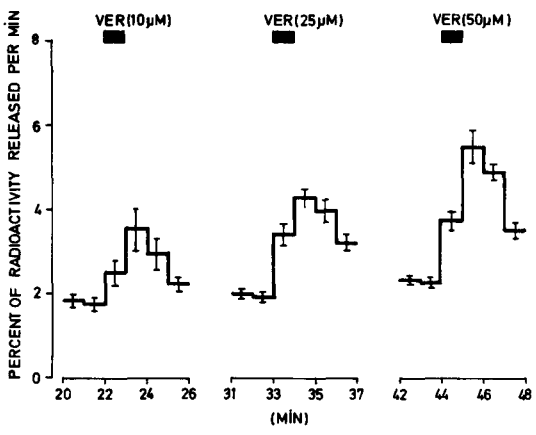

Fig. 1. Effect of increasing concentrations of veratridine on the release of L-[^{3}H]glutamic acid from striatal slices. Striatal tissue slices were incubated with L-[^{3}H]glutamate for 30 min at 37° prior to being placed in superfusion chambers as described in Materials and Methods. Superfusion time is plotted on the abscissa and on the ordinate is the percent of radioactivity released per min of superfusion. Each black horizontal bar represents the period of stimulation by veratridine. The striatal tissue took up 1,561,500 ± 122,182 cpm of L-[^{3}H]glutamate. Results represent the mean ± S.D. of four different experiments.

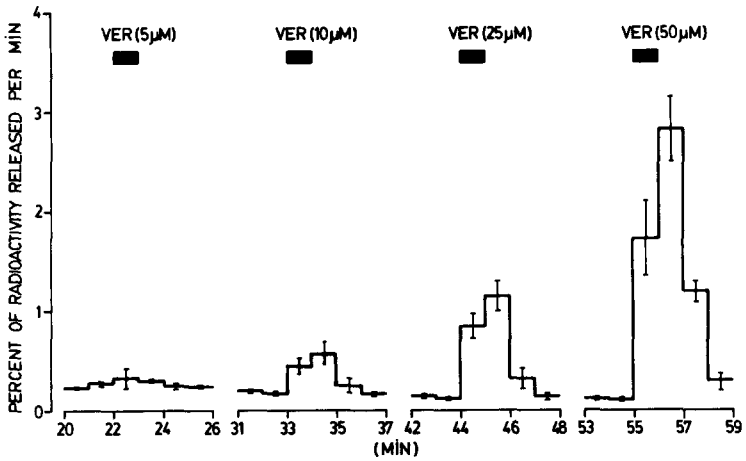

Fig. 2. Effect of increasing concentrations of veratridine on the release of D-[^{3}H]aspartic acid from striatal slices. Tissue slices were incubated with D-[^{3}H]aspartic acid and thereafter superfused as described in Materials and Methods. Superfusion time is plotted on the abscissa and on the ordinate appears the percent of radioactivity released per min of superfusion. Veratridine stimulation is shown by a horizontal bar and was carried out for 1 min after a washout period of 22 min. The striatal slices took up 3,295,750 ± 206,679 cpm of D-[^{3}H]aspartate. Results represent the mean ± S.D. of four different experiments.

Following the incubation period the slices were transferred to a superfusion system in order to measure spontaneous and veratridine-evoked release of the previously taken up ^{3}H-amino acids. As shown in Figs. 1 and 2, spontaneous levels of L-[^{3}H]glutamic acid release were quite high relative to that of D-[^{3}H]aspartic acid. For example, the percentage of radioactivity spontaneously released 1 min before 50 μM veratridine amounted to 2.32 ± 0.18 (mean ± S.E.M.; N = 4) for the L-[^{3}H]glutamic acid experiment (Fig. 1), whereas it only amounted to 0.18 ± 0.02 for the D-[^{3}H]aspartic acid experiment (Fig. 2).

The addition of veratridine (1 min) to the superfusion medium resulted in reversible and dose-dependent increases in L-[^{3}H]glutamic and D-[^{3}H]-aspartic acid release from striatal slices (Figs. 1 and 2). A substantial increase in release occurred with veratridine concentrations as low as 10 μM. The patterns and time-courses of release were similar for both amino acids, as the larger proportion of both L-[^{3}H]glutamic and D-[^{3}H]aspartic acid release occurred 1 min following the stimulation period. In terms of percentage of radioactivity initially accumulated in the slices, the veratridine-evoked releases of label were of similar magnitude for the two amino acids studied (Figs. 1 and 2). However, differences are apparent when the results are expressed relative to the pre-stimulation period. Veratridine (50 μM) depolarization produced a 3.55 ± 0.60 (mean ± S.E.M.; N = 4; $P < 0.005$) fold increase in L-[^{3}H] glutamic acid release over the pre-stimulation period (Fig. 1). In contrast, the same depolarizing stimulus induced a 52.5 ± 5.7 ($P < 0.001$) fold increase of D-[^{3}H]aspartic acid release over spontaneous levels of release (Fig. 2).

Tetrodotoxin (TTX), which blocks the voltage-dependent Na^+ channels, was used to test the specificity of the veratridine-induced release. TTX (1.0 μM) added to the superfusion medium, 12 min before stimulation, totally abolished the increase in D-[^{3}H]aspartic acid release evoked be veratridine (Fig. 3). The inhibition by TTX proved to be partially reversible since, following a 13-min washout period, veratridine was able to again evoke D-[^{3}H]aspartic acid release.

Calcium dependence of depolarization-induced release of D-[^{3}H]*-aspartic acid from striatal slices.* Omission of Ca^{2+} from the superfusion medium had quite different effects on the release of D-[^{3}H]aspartic acid induced by various depolarizing conditions. Omitting Ca^{2+} from the medium and adding 0.1 mM ethyleneglycolbis(amino-ethylether)tetra acetate (EGTA) reduced the K^+-evoked release of the ^{3}H-amino acid by approximately 33% ($P < 0.05$), whereas the release induced by veratridine was blocked 72% ($P < 0.002$) (Fig. 4). In contrast, the release of D-[^{3}H]aspartic acid evoked by electrical field stimulation was almost totally suppressed by the absence of Ca^{2+} (Table 1). When the Mg^{2+} concentration in the Ca^{2+}-free medium was increased to 12 mM, the release induced by K^+-depolarization was reduced 70% and that induced by veratridine was suppressed ($P < 0.001$ in both cases) (Fig. 4).

Effect of fronto-parietal cortex ablation on depolarization-evoked release of D-[^{3}H]*aspartic acid from striatal slices.* Three weeks after fronto-parietal unilateral cortex ablation there was a significant 29% reduction in D-[^{3}H]aspartic acid uptake by slices prepared from the ipsilateral striatum as compared to normal contralateral striatal slices (23.12 ± 0.85 vs 32.15 ± 1.19 pmoles of D-[^{3}H]aspartic acid taken up per mg protein; N = 5; $P < 0.001$). Parallel with these changes a striking reduction in D-[^{3}H]aspartic acid release was observed in response to neuronal depolarization when the release data were expressed as counts per minute of superfusion. As shown in Table 2, potassium- (53 mM) and veratridine- (25 μM) evoked release of ^{3}H-amino acid from ipsilateral striatal slices was reduced, respectively, by

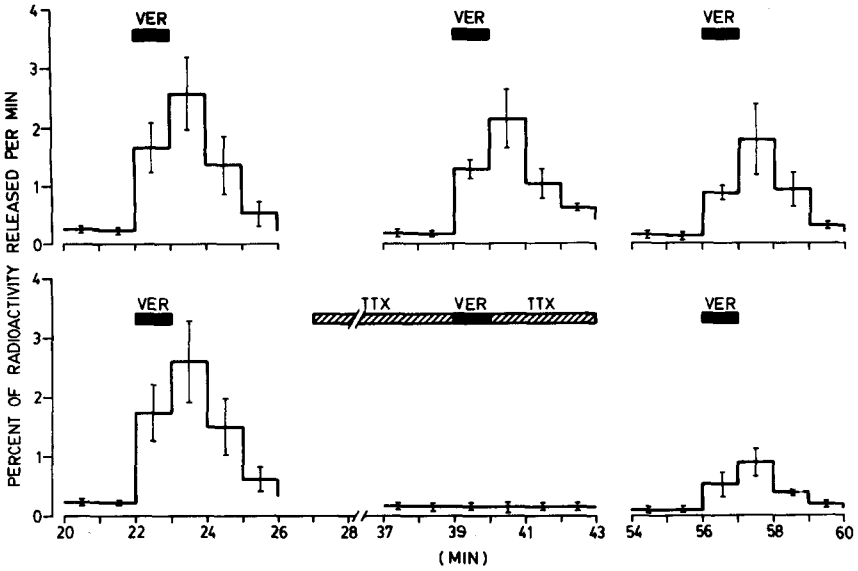

Fig. 3. Effect of tetrodotoxin (TTX) on veratridine-induced release of D-[^{3}H]aspartic acid from rat striatal slices. Tissue slices were incubated with D-[^{3}H]aspartic acid and thereafter superfused as described in Materials and Methods. Veratridine (25 μM) stimulation was carried out for 1.0 min. In the experiments with TTX, the toxin (1 μM) was added to the superfusion medium (hatched areas) 12 min before and throughout the stimulation period (black area). The tissue took up 3,305,870 ± 184,752 cpm and 3,386,451 ± 196,606 cpm of D-[^{3}H]aspartic acid for the veratridine and veratridine plus TTX experiments respectively. On the abscissa is plotted the superfusion time and on the ordinate is the percent of radioactivity released. The figure shows the mean ± S.D. of three different experiments.

20% and 49% ($P < 0.001$ in both cases) when compared to contralateral striatal slices. In contrast, no significant difference was found, between the ipsilateral and contralateral striatum, when the release data were expressed as a percentage of total radioactivity accumulated by the striatal slices.

Effect of striatal lesions with kainic acid on depolarization-evoked release of D-[^{3}H]*aspartic acid from striatal slices.* Eight days after striatal kainic acid lesions there was a marked 85% reduction ($P < 0.001$) in the activity of the enzyme choline acetyltransferase (data not shown), which is a good marker for neuronal cell bodies located in the striatum [32]. In contrast, no significant changes were found in the ipsilateral striatum when compared with the contralateral unlesioned striatum in regard to D-[^{3}H]aspartate uptake by slices (29.52 ± 1.24 vs 32.34 ± 1.64 pmoles of D-[^{3}H]aspartic acid taken up per mg protein; N = 5) and in regard to spontaneous and K^+- (53 mM) induced release of D-[^{3}H]aspartic acid (Table 3).

DISCUSSION

The experiments described illustrate that short periods of veratridine depolarization caused a significant increase in the efflux of labeled L-glutamic and D-aspartic acid from striatal slices (Figs. 1 and 2). The spontaneous release of L-[^{3}H]glutamic acid was 12-fold higher than the resting release of D-[^{3}H] aspartic acid. As a consequence, the ratio of D-[^{3}H] aspartic acid release induced by veratridine over spontaneous levels of release was much higher than the ratio of labeled L-glutamic acid to control. These findings, plus the fact that D-aspartic acid metabolizes very slowly [22, 23], suggest that the accumulation of this amino acid in striatal slices, compared with L-glutamic acid occurred mainly in a functional compartment related to release rather than to metabolic functions. Our findings support the view that labeled D-aspartic acid may be a more selective marker for putative glutamatergic and/or aspartergic nerve terminals than L-[^{3}H]glutamic acid itself.

In this paper, a series of experiments have been described using veratridine and electrical field stimulation, as the effects of these depolarizing conditions are presumably confined to the electrically excitable cells of the nervous [14, 41]. Under our experimental conditions, both veratridine and electrical field stimulation caused a marked increase in D-[^{3}H]aspartic acid release from striatal slices, which was almost totally suppressed in a Ca^{2+}-free medium (Fig. 4 and Table 1). In contrast, omission of Ca^{2+} from the superfusion medium had a rather poor effect on release evoked by K^+ depolarization. In fact, only 30% of the K^+-evoked release of D-[^{3}H]aspartic acid was found to be Ca^{2+} dependent (Fig. 4). These last results agree with recent findings by Mitchell and Doggett [42] in which the K^+-induced release of L-[^{3}H]glutamic acid previously taken up by striatal prisms was found to be inhibited only partially by the absence of Ca^{2+}. The rather poor Ca^{2+} dependence of K^+-depolarization-induced release of D-[^{3}H]aspartic acid suggests that this type of depolarization is not an adequate experimental tool to study stimulus-secretion coupling of putative amino acid transmitters from nerve endings. Besides, K^+-depolarization may evoke the release of amino acids not from nerve endings but, rather, from glial structures [14, 15]. In contrast, the release of ^{3}H-amino

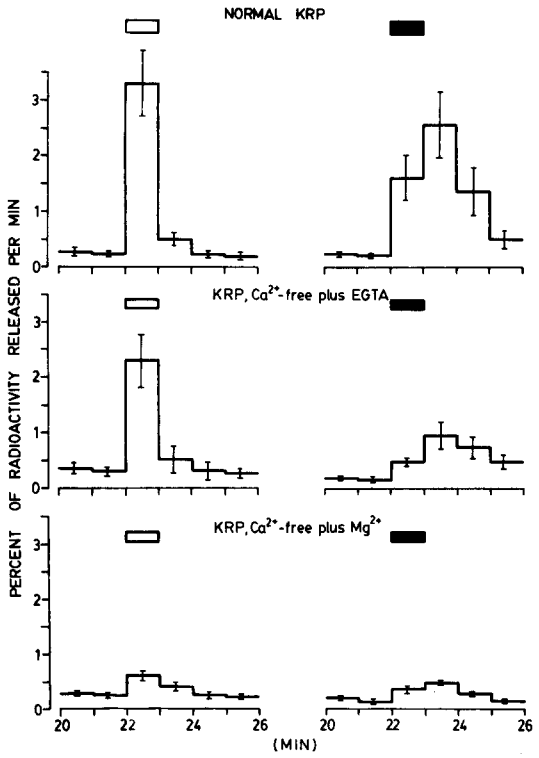

Fig. 4. Calcium dependence of striatal D-[^{3}H]aspartic acid release evoked by high potassium (left graph) and veratridine (right graph). Striatal tissue slices were incubated with D-[^{3}H]aspartic acid for 30 min at 37° and thereafter superfused as described in Materials and Methods. The response to high K^+ (53 mM, □) and veratridine (25 μM, ■) was determined following a 22-min washout period which was performed either with normal KRP; KRP, Ca^{2+}-free + EGTA (1×10^{-4} M); or KRP, Ca^{2+}-free + Mg^{2+} (12 mM). The percent of radioactivity released is plotted on the ordinate and the time of superfusion on the abscissa. The tissue took up 3,562,857 ± 198,158 cpm and 3,205,908 ± 191,582 cpm of D-[^{3}H]aspartic acid for the veratridine and potassium experiments respectively. Results represent the mean ± S.D. of three different experiments under each experimental condition

acids evoked by electrical stimulation and veratridine appeared to be much more Ca^{2+} dependent than K^+ induced release, and it seems, then, that the former depolarizing stimuli used in our studies promote Ca^{2+} influx that, in turn, triggers secretion from striatal structures of the previously taken up D-[^{3}H] aspartic acid. In addition, D-[^{3}H]aspartic acid release induced by veratridine stimulation was completely prevented by tetrodotoxin (Fig. 3). This last finding supports the view that D-aspartate release, evoked by veratridine, comes mainly from nerve terminals located in the striatum, as tetrodotoxin-sensitive Na^+ channels are not believed to be expressed on glial structures [43].

One interesting observation was the fact that veratridine elicited D-aspartic and L-glutamic acid release from striatal slices according to a time-course and pattern of release which were similar for both amino acids (Figs. 1 and 2). In our opinion, these results, plus the findings discussed above, support the idea that veratridine-evoked release of D-aspartic acid occurs from striatal neuronal compartments related to L-glutamic or L-aspartic acid release.

The question remains as to the nature of the neuronal compartment in the striatum upon which depolarizing stimuli act to induce D-[^{3}H]aspartic acid release. To answer this question we have studied release of D-[^{3}H]aspartic acid in the striatum following frontal cortex ablation. Unilateral ablation of the fronto-parietal cortex destroys the cortico-striatal neuronal pathway and, in addition, produces a series of parallel biochemical changes in the striatum, i.e. a decrease in the high-affinity uptake of L-[^{3}H]glutamate and D-[^{3}H]aspartate [5, 6, 24], a specific fall in the L-glutamic acid content, but not L-aspartic acid, in the ipsilateral striatum [4, 44], and a decrease in the Ca^{2+}-dependent release of endogenous striatal glutamate [9]. These findings have given rise to the suggestion that the cortico-striatal pathway predominantly stores and releases excitatory amino acids from its nerve terminals. In agreement with this suggestion, our results show that destruction of fronto-parietal cortex was accompanied by a significant decrease in high-affinity uptake of D-[^{3}H] aspartic acid and by a large loss, from striatal slices

Table 1. Release of D-aspartic acid induced by electrical field stimulation and its calcium dependence*

Stimulation condition	% Release of D-[^{3}H]aspartic acid taken up by striatal slices	
	Normal KRP	KRP, Ca^{2+}-free + EGTA (1×10^{-4} M)
None (spontaneous release)	0.21 ± 0.02	0.20 ± 0.02
Electrical field stimulation (20 Hz, 1.0 V, 60 sec)	0.77 ± 0.14†	0.13 ± 0.04

* Striatal slices were incubated with D-[^{3}H]aspartic acid and superfused as described in Materials and Methods. The electrical field stimulation was carried out for 1 min, in biphasic pulses of 5 msec duration, at a frequency of 20 Hz and 1.0 V after 17 min of washout period. Stimulus-induced release corresponds to percent of D-[^{3}H]aspartic acid release above spontaneous levels of release. The values for the spontaneous release of D-[^{3}H]asparic acid before electrical stimulation were 3,775 ± 342 cpm in normal KRP and 3,612 ± 318 cpm in KRP, Ca^{2+}-free + EGTA (1×1^{-4} M) respectively. The table presents the mean ± S.E.M. of four different experiments. The striatal slices had taken up 3,261,405 ± 195,805 cpm and 3,315,207 ± 208,552 cpm of D-[^{3}H]aspartic acid for the normal KRP and KRP, Ca^{2+}-free experiments respectively. Significance of difference was determined by Student's *t*-test.

† $P < 0.01$ when compared to spontaneous values of release.

Table 2. Effect of fronto-parietal cortex ablation on D-[^{3}H]aspartic acid release from striatal slices*

Unilateral cortical ablation	Release of D-[^{3}H]aspartic acid taken up by striatal slices (cpm/min of superfusion)			
	Spontaneous	Veratridine (25 μM)	Spontaneous	Potassium (53 mM)
Contralateral striatum	5,988 ± 457 (0.17 ± 0.01)	126,818 ± 12,693 (3.70 ± 0.31)	8,614 ± 1,550 (0.26 ± 0.05)	99,996 ± 4,318 (3.07 ± 0.13)
Ipsilateral striatum	4,705 ± 247 (0.23 ± 0.02)	65,104 ± 4,390† (3.16 ± 0.24)	6,730 ± 792 (0.33 ± 0.04)	81,712 ± 2,378† (3.98 ± 0.12)

* Animals were decorticated and killed 21 days later. Striatal slices (7.0 mg) were incubated in 2 ml of KRP for 30 min at 37° in the presence of D-[^{3}H]aspartic acid and then transferred to a superfusion system. Veratridine and potassium stimulation was carried out for 1 min after 22 min of washout period. Stimulus-induced release corresponds to cpm of D-[^{3}H]aspartic acid released above spontaneous levels of release. Release was measured in the ipsilateral and contralateral striatum which served as control. The striatal slices had taken up the following amounts of radilabeled D-aspartate: contralateral striatum, veratridine, 3,437,427 ± 182,405 cpm; ipsilateral striatum, veratridine, 2,062,456 ± 114,915; contralateral striatum, high K^+, 3,261,508 ± 191,305; ipsliateral striaum, high K^+, 2,054,750 ± 130,087. The numbers in parentheses indicate the data expressed as percentage of total radioactivity taken up by the slices. Each value is the mean ± S.E.M. of five different experiments. Significance of difference was determined by Student's *t*-test.

† $P < 0.001$ when compared to respective contralateral control striatum.

of ipsilateral to the lesion, in D-[^{3}H]aspartic acid release evoked by veratridine and high K^+ (Table 2). However, the loss in D-[^{3}H]aspartate release from the ipsilateral striatum was only apparent when the release data were expressed as counts per minute of superfusion and not when they were expressed as percentage of total radioactivity taken up by the slices. Therefore, the decrease in release after decortication seems to directly reflect the reduction in cortico-striatal nerve terminals, rather than the reduction in release *per se*. The loss of veratridine-evoked release of ^{3}H-amino acids was greater than the loss of K^+-evoked release in agreement with the fact that the former depolarizing condition is confined to electrically excitable cells of the nervous system [41]. All these findings taken together favor the idea that, under our experimental conditions, D-[^{3}H]aspartic acid is preferentially taken up and then released from nerve terminals of the cortico-striatal pathway. Additional evidence supporting this idea has been provided by experiments in which the effects of intrastriatal kainic acid injection on D-aspartic induced release were studied (Table 3). If D-[^{3}H]aspartic acid were accumulated and released mainly from striatal nerve afferences, one would expect little if any change in the stimulus-evoked release of D-[^{3}H]aspartic acid following kainic acid lesion. In confirmation of this prediction, we found negligible changes in the high-affinity uptake of D-[^{3}H]aspartic acid and a non-significant decline in depolarization-induced release of D-[^{3}H]aspartic acid in slices obtained from ipsilateral kainic acid injected striatum.

To the best of our knowledge, we are among the first to used D-[^{3}H]aspartate as a selective marker to study evoked amino acid release from striatal tissue [30, *]. In agreement with our results, Potashner and Gerard [31] have reported recently that electrical stimulation of guinea pig striatal slices evoked a Ca^{2+}-dependent release of D-[^{3}H]aspartate. However, D-[^{3}H]aspartate has been used quite extensively as a marker for evoked amino acid release in other

* L. Arqueros, J. Abarca and G. Bustos, Abstr., Ninth Latinoamerican Congress of Pharmacology and Therapeutics, Oct. 17–23, Santiago, p. 88 (1982).

Table 3. Effect of striatal lesions with kainic acid on D-[^{3}H]aspartic acid release from striatal slices*

Striatal lesions with kainic acid	Release of D-[^{3}H]aspartic acid taken up by striatal slices (cpm/min of superfusion)	
	Spontaneous release	K^+-induced release
Contralateral striatum	7,382 ± 877	80,180 ± 3,900
Ipsilateral striatum	8,383 ± 1,220	76,781 ± 9,749†

* Rats were injected stereotaxically in the left striatum with 2 μg kainic acid in 1 μl KRP and then killed 8 days later. Striatal slices (7.0 mg) were incubated in KRP for 30 min at 37° in the presence of D-[^{3}H]aspartic acid and then transferred to a superfusion system. Potassium stimulation (53 mM; 1 mM) was carried out following a 22-min washout period. Release was measured in the ipsilateral and contralateral striatum which served as control. Slices prepared from contralateral and ipsilateral striatum had taken up 3,234,508 ± 206,879 cpm and 3,217,681 ± 508,915 cpm of D-[^{3}H]aspartic acid, respectively. Results represent the mean ± S.E.M. of five different experiments.

† Not significantly different when compared to respective contralateral control striatum.

brain regions; for example, Malthe-Sorenssen *et al.* [25, 26] reported a Ca^{2+}-dependent electrically evoked release of D-[^{3}H]aspartate from afferent fibers to hippocampal pyramidal tracts and from afferents from fimbria to dorsolateral septum.

In conclusion, our results indicate that D-[^{3}H] aspartic acid was accumulated by nerve terminals located in the striatum and was released from them, following veratridine and electrical field stimulation, in a Ca^{2+}-dependent manner. Cortical ablation experiments and striatal lesions with kainic acid further suggested that these D-[^{3}H]aspartic acid-accumulating nerve terminals may belong to the cortico-striatal neuronal pathway. Finally, our results support the view that amino acids such as L-glutamate and L-aspartate can be regarded as transmitter candidates for cortico-striatal nerve terminals.

Acknowledgements—We wish to express our thanks to Ms Lucy Chacoff and Jenny Fiedler for their help in the preparation of the manuscript. This work was supported by grants from "Dirección de Investigaciones Universidad Católica", from "Pro-Funcación Reciporcidad", and from "Fondo Nacional de Desarrollo Científico y Tecnológico".

REFERENCES

1. C. W. Cotman, A. Foster and T. Lanthorn, in *Glutamate as a Neurotransmitter* (Eds. G. Di Chiara and G. L. Gessa), p. 1. Raven Press, New York (1981).
2. G. E. Fagg and A. C. Foster, *Neuroscience* **9**, 701 (1983).
3. K. E. Webster, *J. Anat.* **95**, 532 (1961).
4. J. S. Kim, R. Hassler, P. Haug and K. S. Paig, *Brain Res.* **132**, 370 (1977).
5. P. L. McGeer, E. G. McGeer, U. Scherer and K. Singh, *Brain Res.* **128**, 369 (1977).
6. F. Fonnum and J. Storm-Mathisen, *Nature, Lond.* **266**, 377 (1977).
7. J. C. Reubi and M. Cuenod, *Brain Res.* **176**, 185 (1979).
8. J. C. Reubi, *Neuroscience* **5**, 2154 (1980).
9. G. J. Rowlands and P. J. Roberts, *Expl. Brain Res.* **39**, 239 (1980).
10. V. Srinivasan, M. J. Neal and J. F. Mitchell, *J. Neurochem.* **16**, 1235 (1980).
11. D. A. Redburn, D. Shelton and C. W. Cotman, *J. Neurochem.* **26**, 297 (1976).
12. J. W. Haycock, W. B. Levy, L. A. Denner and C. W. Cotman, *J. Neurochem.* **30**, 1113 (1978).
13. J. P. Hammerstad, M. L. Cawthon and C. R. Lytle, *J. Neurochem.* **29**, 195 (1979).
14. F. Orrego, *Neuroscience* **4**, 1037 (1979).
15. M. C. W. Minchin and L. L. Iversen, *J. Neurochem.* **23**, 533 (1974).
16. S. J. Potashner, *J. Neurochem.* **31**, 177 (1978).
17. S. J. Potashner, *J. Neurochem.* **31**, 187 (1978).
18. M. Ohta, T. Narahashi and R. F. Keeler, *J. Pharmac. exp. Ther.* **184**, 143 (1973).
19. M. P. Blaustein, *J. Physiol. Lond.* **247**, 617 (1975).
20. P. P. Li and T. D. White, *J. Neurochem.* **28**, 967 (1977).
21. V. J. Balcar and G. A. R. Johnston, *J. Neurochem.* **19**, 2657 (1972).
22. L. P. Davies and G. A. R. Johnston, *J. Neurochem.* **26**, 1007 (1976).
23. G. Takagaki, *J. Neurochem.* **30**, 47 (1978).
24. F. Fonnum, J. Storm-Mathisen and I. Divac, *Neuroscience* **6**, 863 (1981).
25. D. Malthe-Sorenssen, K. K. Skrede and F. Fonnum, *Neuroscience* **4**, 1255 (1979).
26. D. Malthe-Sorenssen, K. K. Skrede and F. Fonnum, *Neuroscience* **5**, 127 (1980).
27. A. Beaudet, A. Burkhalter, J. C. Reubi and M. Cuenod, *Neuroscience* **6**, 2021 (1981).
28. D. L. Oliver, D. R. Jones, S. J. Potashner and D. K. Morest, *Anat. Rec.* **199**, 186A (1981).
29. L. Wiklund, G. Toggenburg and M. Cuenod, *Science* **216**, 78 (1982).
30. L. Arqueros and J. Abarca, *Arch. Biol. Med. exp.* **14**, 249 (1981).
31. S. J. Potashner and D. Gerard, *J. Neurochem.* **40**, 1548 (1983).
32. R. Schwarcz and J. T. Coyle, *Brain Res.* **127**, 235 (1977).
33. J. F. R. König and R. A. Klippel, *The Rat Brain—A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Krieger, Huntingdon, New York (1963).
34. B. K. Schrier and L. Shuster, *J. Neurochem.* **14**, 977 (1967).
35. L. Arqueros and J. Abarca, *Arch. Biol. Med. exp.* **12**, 496 (1979).
36. R. Laverty and K. M. Taylor, *Analyt. Biochem.* **22**, 269 (1968).
37. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
38. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
39. G. Bustos and R. H. Roth, *Br. J. Pharmac.* **46**, 101 (1972).
40. M. I. Rudolph, L. Arqueros and G. Bustos, *Neurochem. Int.* **5**, 479 (1983).
41. W. Ulbricht, *Biophys. Struct. Mechanism* **1**, 1 (1974).
42. P. R. Mitchell and M. S. Doggett, *Life Sci.* **26**, 2073 (1980).
43. A. M. Benjamin and J. H. Quastel, *Biochem. J.* **128**, 631 (1972).
44. R. Hassler, P. Haug, C. Nitsch, J-S. Kim and K. Paik, *J. Neurochem.* **38**, 1087 (1982).