Substantia nigra osmoregulation: taurine and ATP involvement

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Mora les I, Dopico JG, Sab ate M, Gon zalez-Hernandez T, Rod ri guez M. Substantia nigra osmoregulation: taurine and ATP involvement. Am J Physiol Cell Physiol 292: C1934–C1941, 2007. First published January 10, 2007; doi:10.1152/ajpcell.00593.2006.—An extracellular nonsynaptic taurine pool of glial origin was recently reported in the substantia nigra (SN). There is previous evidence showing taurine as an inhibitory neurotransmitter in the SN, but the physiological role of this nonsynaptic pool of taurine has not been explored. By using microdialysis methods, we studied the action of local osmolarity on the nonsynaptic taurine pool in the SN of the rat. Hypoosmolar pulses (285–80 mosM) administered in the SN by the microdialysis probe increased extrasynaptic taurine in a dose-dependent way, a response that was counteracted by compensating osmolarity with choline. The opposite effect (taurine decrease) was observed when osmolarity was increased. Under basal conditions, the blockade of either the AMPA-kainate glutamate receptors with 6-cyano-7-nitroquinoxaline-2,3-dione disodium or the purinergic receptors with pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid modified the taurine concentration, suggesting that both receptors modulate the extrasynaptic pool of taurine. In addition, these drugs decreased the taurine response to hypoosmolar pulses, suggesting roles for glutamatergic and purinergic receptors in the taurine response to osmolarity. The participation of purinergic receptors was also supported by the fact that ATP (which, under basal conditions, increased the extrasynaptic taurine in a dose-dependent way) administered in doses saturating purinergic receptors also decreased the taurine response to hypoosmolarity. Taken together, present data suggest osmoregulation as a role of the nonsynaptic taurine pool of the SN, a function that also involves glutamate and ATP and that could influence the nigral cell vulnerability in Parkinson’s disease.

MATERIALS AND METHODS

Experiments were carried out on male Sprague-Dawley rats weighing 300–350 g. Animals were housed at 22°C, two per cage, under normal laboratory conditions on a standard light-dark schedule (12:12 h, with 0300 to 1500 lights on) and with free access to food and water. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) regarding the care and use of animals for experimental procedures, and adequate measures were taken to minimize pain and discomfort. Experimental protocols were reviewed and approved by the Ethical Committee for Research at La Laguna University.

Microdialysis. Microdialysis was used according to previously reported procedures (28, 73). Animals were anesthetized with equitensine (pentobarbital sodium 29 mg/kg, chloral hydrate 127.5 mg/kg, MgSO4 71.4 mg/kg, dihydroxypropane 40%, and ethanol 10%), an anesthetic that does not change the extracellular concentration of amino acids (75). To maintain a long-lasting, stable anesthesia,
animals were continuously intraperitoneally (ip) perfused with equitensine (0.5 ml/h) beginning 60 min after the initial ip administration of the anesthetic. Body temperature was monitored and maintained between 36.5 and 37.0°C for the whole session. A concentric microdialysis probe (220-μm diameter, 1-mm long, and in vitro recovery of ~15% for amino acids) was introduced in the SN (3.0 mm anterior to lambda, 2.0 mm lateral to the midline, and 8.0 mm under the cortical surface) and perfused with a Ringer solution (NaCl 148 mM, KCl 2.7 mM, CaCl2 1.2 mM, and MgCl2 0.8 mM) at a rate of 2.0 μl/min. After samples from the first 150 min were discarded, dialysate fractions were collected at 10-min intervals. The dependence of extracellular taurine on osmolarity was studied by perfusing hypoosmolar or hyperosmolar solutions through the dialysis membrane during short time periods (pulses of 5 min). Two basal conditions were evaluated, one with a normal osmolarity (285 mosM) and the other with low osmolarity (230 mosM). The osmolarity of Ringer preparations was quantified with an autoosmometer (Osmostat OM-6020; CagaK, Kyoto, Japan). Ringer solutions were modified by progressively decreasing (to obtain Ringer solutions of 230, 180, 130, and 110 mosM) or increasing (to obtain solutions of 300 mosM) the NaCl concentration. To distinguish the possible influence of the Na⁺/Cl⁻ low concentration from the hypoosmolarity effects in hypoosmolar solutions, a 60 mM choline-Cl was occasionally added to the 180 mosM Ringer solution (91), which therefore had a low ionic concentration but an osmolarity similar to that of basal Ringer solutions (285 mosM).

The influence of ionotropic glutamate receptors on basal extrasynaptic taurine was studied by perfusing an α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)-kainate receptor antagonist (6-cyano-7-nitroquinoxaline-2,3-dione disodium, CNQC; 500 μM, Tocris) with reverse microdialysis. The influence of these receptors on the taurine response to hypoosmolarity was studied by comparing the taurine response to hypoosmolar pulses of 5 min (130 mosM vs. a basal osmolarity of 285 mosM) administered before and after pretreatment with CNQC (500 μM perfused starting from 40 min before the second hypoosmolar pulse).

The influence of purinergic receptors on basal extrasynaptic taurine was studied by quantifying the taurine concentration before and after administration of a receptor agonist (adenosine 5'-triphosphate standard disodium salt hydrate, ATP; Sigma-Aldrich, Madrid, Spain) or a receptor antagonist (pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid, PPADS, which is a P2 receptor antagonist; 1 mM, Tocris) with reverse microdialysis. A concentration-response curve was drawn for ATP studies for 5-min pulses of ATP solutions (0.1, 1, 10, 50, and 250Fig. 1. Taurine and nigral cell adaptation to hypoosmotic conditions. A: dose-dependent increase (285-130 mosM) of extrasynaptic taurine during hypoosmotic Ringer perfusion. B: inhibition of taurine response by substituting the NaCl omitted in the perfusing solution with choline-Cl. C: taurine response to marked hypoosmolar pulses (80 mosM) after decreasing toxicity below a normal (285 mosM at left) and below a low osmolarity (210 mosM at right) basal Ringer solution. D: decrease of extrasynaptic taurine after increasing toxicity above a normal (285 mosM at left) and above a hypotonic (230 mosM at right) basal Ringer solution. Values are percentages of the basal levels 10 min before the hypoosmolar pulse administration (mean ± SE).

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The influence of purinergic receptors on the taurine response to hypoosmolarity was studied by comparing the taurine response to hypoosmolar pulses of 5 min (180 mosM vs. a basal osmolarity of 285 mosM) administered before (starting from 40 min before the second hypoosmolar pulse) and during PPADS (1 mM) or ATP (perfused in saturating concentrations of 100 μM) administration.

Biochemical analyses. Taurine and glutamate were determined on a high-performance liquid chromatograph (HPLC) system with a 4-μm C18 reverse-phase column (100 x 4.6 mm) and fluorimetric detection. Precolumn derivatization was performed with o-phthalaldehyde-2-mercaptoethanol-borate reagent (pH 10.4). Dialysate (10 μl) was derivatized with the same volume of the o-phthalaldehyde reagent solution (P0532; Sigma, St. Louis, MO), and, after a 120-s reaction period (6°C in a 717plus autosampler; Waters), the reaction was stopped with 5 μl of acetic acid (5%) and immediately injected into a HPLC. The mobile phase (pH 5.65) was composed of sodium acetate (0.05 M), methanol (5%), and isopropyl alcohol (1.36%) and flowed at 1.0 ml/min in a gradient (20-min duration) from 25 to 75% methanol (48). The fluorimetric detector (model no. 474; Waters) had the excitation wavelength set at 370 nm and the emission cut-off filter set at 450 nm. The recovery rate of the microdialysis probe was tested in vitro at the start of each experiment for the posterior calculation of the extracellular concentration of taurine.

Histology. At the end of each experiment, the rats were transcardially perfused with 200 ml of 0.9% saline solution followed by 400 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) to confirm the microdialysis probe placement. Brains were removed and stored in the same fixative at 4°C for 12–24 h, and then the midbrain was cut at 50 μm with a vibratome in the coronal plane and stained with the formal thionine procedure.

Statistics. Mathematical analyses were performed using one-way ANOVA followed by the least significant difference test for post hoc comparisons. Analysis was performed using the Statistica program (Statsoft). A level of P < 0.05 was considered as critical for assigning statistical significance.

RESULTS

The extracellular taurine concentration observed here with microdialysis in the substantia nigra was 2.7 ± 0.45 μM.

In the first experiment, a group of seven rats was administered with three successive pulses (5 min/pulse) of low osmolar Ringer (Fig. 1A). Hypoosmolarity increased extrasynaptic taurine concentration in a dose-dependent way, inducing a 60% increase after the first pulse (230 mosM) and a 550% increase after the third pulse (130 mosM). Except for the highest stimulus (130 mosM), which showed a small taurine increase in the dialysate obtained 5–15 min after the hypoosmolar pulse switch-off, the taurine response was observed only in the dialysate obtained during stimulation, having completely disappeared 5 min after the stimulus switch-off.

In the second experiment, rats (n = 6) were administered with two successive hypoosmolar pulses: the first one was performed with a low NaCl concentration (88 mM) to obtain a osmolarity of 180 mosM, and the second one had the same low NaCl concentration, but its osmolarity was restored to that of normal Ringer (275 mosM) by adding choline-Cl (60 mM). The first pulse increased the taurine concentration, an effect that vanished when the hypoosmolarity was compensated with choline-Cl (Fig. 1B). This showed that the taurine increase induced by low osmolarity is a specific phenomenon not induced by the low concentration of sodium or chloride.

In the third experiment, two groups of rats (6 rats each) were administered with marked hypoosmolar pulses (80 mosM), but, whereas in the first one, the osmolarity of the Ringer solution perfused under basal conditions was in the physiological range (285 mosM), in the second one, it was clearly below the normal level (210 mosM). Taurine increased in both cases (Fig. 1C), but the response was higher in the group that started from a normal basal osmolarity (the difference between the basal and pulse osmolarity was a 200 mosM difference) than in the low osmolarity basal group (130 mosM difference). This showed that the taurine response to hypoosmolarity also remains active when the basal osmolarity is very low.

In the fourth experiment, the basal osmolarity of two rat groups (of 5 rats each) was slightly increased, but, whereas in the first one, the Ringer perfused under basal conditions was in the physiological range (285 mosM), in the second one, it was clearly below normal levels (245 mosM). In both cases, the osmolarity increase during the pulse was 15 mosM (from 285 to 300 mosM in the first case, and from 230 to 245 mosM in the
second one). The hyperosmolar pulse decreased the extracellular taurine to ~70–80% of its basal level in both groups (Fig. 1D), suggesting that taurine also responds to osmolarity increases, even when they are small increases around normal osmolarity levels.

The fifth experiment showed an osmosensitive response for nigral extrasynaptic glutamate. A 300% increase of extrasynaptic glutamate (Fig. 2A) was observed \( n = 6 \) during the administration of hypoosmotic pulses (130 mosM vs. a basal osmolarity of 285 mosM). In addition, the AMPA-kainite glutamate receptor blockade with CNQC \( n = 9 \) decreased the taurine response (Fig. 2B) to hypoosmolarity pulses (130 vs. 285 mosM). These data suggest that hypoosmolarity facilitates glutamate release, which, acting on ionotrophic glutamate receptors, increases the taurine osmoregulatory response.

The aim of the following experiments was to study the possible involvement of purinergic receptors on the taurine response to hypoosmolarity. Initially, we observed \( n = 8 \) a dose-dependent increase of extracellular taurine in response to ATP-enriched Ringer solutions (Fig. 3A). Thus the amplitude of taurine response was higher when the ATP concentration was increased from 0.1 to 10 \( \mu \)M. Although no further increases were observed for ATP stimuli >10 \( \mu \)M (350% increase), the response duration persisted for longer time periods when the ATP concentration of stimulatory pulses was increased (10 min for 1 \( \mu \)M ATP, 20 min for 10 \( \mu \)M ATP, >40 min for 50–250 \( \mu \)M ATP). When a persistent ATP administration \( n = 5 \) was performed (instead of the short-lasting stimulus previously used), a stable taurine increase was observed (Fig. 3B). In addition, ATP (100 \( \mu \)M) decreased the taurine response to hypoosmolarity [Fig. 3C shows the response to 180 mosM vs. (basal) 285 mosM when osmolar pulses were produced before and during ATP perfusion].

The possible ATP action on taurinergic response to hypoosmolarity was also supported by experiments with PPADS, a purinergic receptor antagonist. PPADS decreased \( n = 6 \)
extrasympathetic taurine (Fig. 4A), suggesting that, under basal conditions of osmolality, purinergic receptors facilitate taurine release. In addition, PPADS \((n=7)\) decreased the taurine response to hypoosmolality [Fig. 4B shows the response to 180 mosM vs. (basal) 285 mosM when osmolar pulses were produced before and during PPADS perfusion], data suggesting that the taurine osmoregulatory response is facilitated by the purinergic receptor activation.

**DISCUSSION**

The present data show a sensitive and proportional response of the extrasympathetic pool of taurine to the extracellular osmolality in the SN, a response facilitated by ATP and glutamate release. These data are the first evidence suggesting an osmoregulatory role for taurine in the SN, a center whose only known function is inhibitory neurotransmission. Bearing in mind the recently reported relevance of inflammation in the SN cell degeneration that characterizes Parkinson’s disease, the osmoregulatory role of taurine reported here suggests that this amino acid is a new variable to be included in the multifactorial puzzle that is now being composed to explain the initiation and evolution of this illness.

The main finding of this study was the dose-dependent increase of extrasympathetic taurine as a result of the perfusion of hypoosmolar solutions (OTR). Although these solutions contained low concentrations of NaCl, the taurine response was not the consequence of the low sodium and chloride levels, as shown by the fact that the taurine response to low ionic solutions disappeared when hypoosmolality was compensated with choline. The opposite effect (taurine decrease) was observed after increasing the osmolarity in the perfusing solutions. The OTR is probably present under both physiological (as suggested by the modifications observed after osmolarity changes of <5%) and pathological (as suggested by the modifications observed after 20–70% osmolarity changes) conditions. Previous studies have reported a taurine release by striatonigral neurons (5, 14), which, acting on glycine receptors (16, 32, 46), modify the chloride conductance (92), the firing activity of SN cells (17), and motor behavior (69). In addition to this neurotransmitter pool, we recently reported a nonsympathetic pool of taurine in the nigra that changed after modifying the glial cell metabolism with fluorocitrate and L-methionine sulfoximide (28). Present data suggest the nigral cell osmoregulation as a function of this extrasympathetic taurine pool (microdialysis is a suitable method for studying extrasympathetic substances that diffuse across the extracellular space but not for studying neurotransmitters that are released and taken up in the synaptic cleft) (11, 35, 87). Thus taurine may have two different functions in the SN, the previously reported function as inhibitory neurotransmitter and the function as extrasympathetic osmotic regulator suggested here.

Although taurine is one of the most studied osmolytes, the molecular identity of the membrane permeability pathways used by taurine to leave the cells during the osmotic response is not fully known. Some of mechanisms proposed for taurine outflow are nonselective (e.g., swelling-activated anion channels and hemichannels of gap junctions) (3, 20, 22, 39, 40, 93), thus explaining the fact that taurine release is normally accompanied by the release of other osmolytes (25, 53, 68, 74, 81). This is the case for glutamate and ATP, two substances that, with low molecular masses and high cytosolic concentrations, can cross the cell membrane by some of the pathways proposed for taurine outflow (e.g., cytosolic molecules of <1 kDa can cross hemichannels) (40, 93). The present finding showing a collateral release of glutamate (and presumably of ATP) accompanying nigral OTR suggests that, in a similar way to what is observed in other cells, taurine response to osmolality involves nonselective membrane pathways in the SN as well.

Taurine response decreased both after PPADS administration and under the action of saturating concentrations of ATP. This, together with the fact that extracellular taurine increased after ATP administration, suggests that a portion of OTR was activated by the osmotic stimulation of ATP release. Previous studies in other cells have reported a two-step osmosensitive release of ATP, an initial release directly associated with hypoosmolality (perhaps across the swelling-activated anion channels) (6, 33, 34) followed by an autocrine activation of purinergic receptors (9, 52, 74, 82), which, after forming large

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**Fig. 4. ATP receptor and taurine response to hypoosmolality.** A: taurine release during the continuous perfusion of 1 mM pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS). B: taurine release during hypoosmolar pulse administration under basal conditions (left) and after pretreatment with 1 mM PPADS (right). Values are percentages of the basal levels 10 min before PPADS or hypoosmolar pulse administration (mean ± SE).

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membrane channels, enhances and prolongs the ATP release (56). Because these channels are permeable to molecules up to 900 Da (56), their opening by ATP facilitates the taurine efflux (4, 67). In addition, the purinergic receptor stimulation can open swelling-activated anion channels (10), which could be an additional mechanism for ATP action on taurine release (4, 67). The self-sustained feedback response to ATP could be at the basis of the persistent taurine release observed here after both the administration of the highest ATP doses (which did not induce a supplementary increase of extracellular taurine but prolonged the time of taurine response) and the administration of hypooosmolar solutions under ATP receptor saturation conditions (after high ATP doses). Thus present data suggest that a portion of nigral OTR involves purinergic receptors. The decreased basal taurine observed after PPADS administration suggests that ATP modulation of taurine release is active under basal conditions (and not only under hypoosmotic conditions), pointing to P2 (highly expressed in the SN) (38) as being the purinergic receptors involved in this ATP action.

Different data also suggested the involvement of glutamate in OTR. In agreement with previous in vitro studies (25, 68, 81), here glutamate increased under hypooosmolar conditions. The mechanisms for osmosensitive glutamate release are not well known, but, bearing in mind the high cytosolic concentration of glutamate (mM in the cytosol vs. μM in the extracellular space) (55, 85), the osmoregulatory opening of non-selective membrane pathways, and the previously reported ATP-glutamate co-release (90), it is possible that the glutamate response to osmosality is a two-step process: the first step is performed by the same nonspecific membrane pathways used for ATP response, and the second step is feedback facilitated by the initial ATP release (18, 23, 26, 27, 71). The glial transporters are very efficient in preventing the glutamate flow across the extracellular medium and its diffusion to the microdialysis probe (61, 62). Thus the glutamate increase observed here suggests that hypooosmolarity induces a large enough glutamate release to saturate the glutamate transporters. In addition, we observed a reduction of OTR after the AMPA/kainite receptor blockade with CNQX. This, together with previous evidence showing an increase of taurine release after glutamate and AMPA administration (28), suggests that the glutamate release induced by hypooosmolarity facilitates OTR.

Recent evidence shows inflammation as a critical factor for SN cell degeneration in PD (31, 86). Although the intra-/extracellular exchange of low molecular mass solutes is the most common and efficient adaptation to swelling, massive efflux of some molecules can damage both the osmolite releasing cells (i.e., the loss of ATP can cause a lack of energy) and their neighboring cells (i.e., the release of glutamate can cause excitotoxicity). This could be especially dangerous for nigral dopaminergic neurons, which are particularly vulnerable to energetic imbalance (8, 77) and to the toxic action of glutamate (57, 60, 64). In this context, the extrasynaptic taurine pool could be particularly suitable for regulating SN cell osmolarity without inducing the collateral effects of other osmolites. Taurine meets the requirements for a biologically ideal osmo-regulator with high water solubility and low lipophilicity (which facilitates a high concentration gradient across cell membranes), a capability of flowing efficiently through membrane channels when needed, and no metabolic functions (greatest proportion of taurine is excreted in an unchanged form and is often considered an inert waste product of the sulfur metabolism) (37). Taurine release directly modulated by osmolarity or indirectly modulated by other osmolites such as ATP or glutamate could perform a key function for dopamine cell survival under unfavorable conditions such as those present in the SN during PD evolution (58, 59).

GRANTS

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