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Alterations of GABA Metabolism and Seizure Susceptibility in the Substantia Nigra of the Kindled Rat Acclimating to Changes in Osmotic State*

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Seizure susceptibility and GABA metabolism were altered in the substantia nigra [SN] of adult male Sprague Dawley rats when these animals were acclimating to an altered plasma osmolality. Changes in GABA metabolism were measured in vivo in SN of the freely moving rat. Suitable precautions were taken to avoid any post-mortem flux of glutamate to GABA and to correct for the underestimation of GABA build up in SN due to the finite diffusion rate of γ-vinyl GABA [GVG] after stereotaxic injection of small amounts into one side of the brain. Control experiments provided evidence that changes in osmolality, within a normal physiological range, did not affect significantly γ-aminobutyric acid transaminase [GABA-T]. Also kindling via the medial septum [MS], in the absence of electrical stimulation did not alter GABA metabolism in SN, thus providing a stable baseline for studies of osmotic effects. Hyperosmolality was associated with a rise in seizure thresholds, with a marked reduction of the rate of GABA synthesis in SN, and with a substantial increase in turnover time of the GABA pool. Hypoosmolality, of a degree known to be associated with mild cerebral edema and swelling localized to astrocytes, markedly reduced seizure threshold, and reduced GABA pool size in SN, but did not alter the rate of GABA synthesis significantly. These results demonstrate by new and independent means the relationship between GABA metabolism in the SN and seizure susceptibility in vivo.

KEY WORDS: GABA metabolism; seizure; kindling, plasma osmolality.

INTRODUCTION

The apparent relationship between osmotic disturbances and neurological function was recognized more than 50 years ago (1). The seizure inducing properties of over-hydration and water intoxication (2) as well as the seizure protecting effect of dehydration (3, 4) were noted. In fact, in an authoritative textbook it is stated that the major clinical manifestations of hypo- and hyper-osmolar states are neurologic (5). Despite the fact that the literature contains many papers describing some intracellular effects of hyperosmolality (6–8) and hypoosmolality (9–11) the exact nature of the metabolic alterations that produce these profound physiological effects in the nervous system are only beginning to be described. The overall status of this area of research has been reviewed recently (12, 13).

The involvement of the GABA system in hyper- and hypo-osmotic disorders has, to date, received little attention. However since neural excitability, convulsive disorders and seizure susceptibility have all been linked

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quite firmly to the GABA system (14–18), it appeared worthwhile to test the involvement of GABA with osmoregulation in a well defined animal model. The exact mechanism by which the GABA system exerts its physiological effects remains unknown. Absolute and relative concentration and metabolic changes in the brain as a whole, regional, cellular (12, 19), subcellular (20, 21) and synaptic level (22, 23) have been invoked. More recently differences in GABA receptors, receptor numbers, receptor sensitivity (24–26), GABA transport, uptake, release (27–29), and the influence of GABA upon gating mechanisms of inorganic ions (30,31) has received most of the attention. The present communication focuses once again upon GABA at the regional metabolic level.

The substantia nigra [SN] plays a major role in seizure mechanisms, as evidenced by its metabolic response to seizures (32) and by its gating role for seizure expression (33). Bilateral injection of GABA agonists into the SN pars reticulata has major anticonvulsant effects (33). However, in spite of the essential role of GABAergic mechanisms in that location, GABA metabolism in SN has not been studied in detail.

In the present study the kindling model of epilepsy in the mature male rat has been used. The effect of short-term osmotic alterations on turnover, concentration, and metabolism of GABA in SN have been studied. Changes in the GABA system have been noted in the substantia nigra which appear essential for the neural network of kindled seizures.

Osmotic changes influence carbohydrate and nitrogen metabolism not only in neuronal tissues but also in the liver (34, 35). GABA concentrations appear to correlate with changes in carbohydrate metabolism (36). Whereas osmotic change in toad and rat has little effect on overall taurine levels in brain tissues, taurine may be of profound importance as an osmoregulator between brain cells and extracellular fluid (37). Hypoosmolality in vivo creates a profound transient change in plasma taurine levels (38) which may, in turn, regulate carbohydrate metabolism via its effect on insulin receptors (39). Using these and more recent findings (40) it has been proposed that taurine may be one possible regulator of GABA metabolism during changes in plasma osmolality (38). Details of this possibility will be presented in a subsequent publication.

EXPERIMENTAL PROCEDURE

Chemicals, Animals, and Methods. Bantin and Kingman Sprague Dawley male rats were used. All were unanesthetized and freely moving and weighed between 300–400 gm at the time of experimentation. γ-vinyl GABA (GVG) was a gift from Merrell Dow Research Institute, Strasbourg, France and from Dr. Karen Gale, Georgetown University, Washington D.C. Arginine vasopressin, HEPES, and 3-mercaptopropionic acid [3MPA] were purchased from Sigma Chemical Co., St. Louis, MO. Ketamine and xylazine were obtained from Aveco, Ft Dodge, IO. and Shawnee, KA. respectively. Biosafe II counting fluid was obtained from Research Products International, Prospect IL. All other reagents used, were reagent grade: Sulfosalicylic acid dihydrate and BCA protein reagent from Pierce Chemical Co., Rockford, IL. OPA reagent and mercaptoethanol from Sigma Co., and potassium tetraborate from Alltech Assoc. Inc., Deerfield, IL. Sodium acetate, methanol, mannitol, and all inorganic salts were Baker analyzed reagents, obtained from Allied Chemicals (Morristown, NJ.).

Preliminary Animal Preparations. For all surgical procedures rats were anesthetized with a mixture of ketamine/xylazine, 6 mg and 1.5 mg/100 g body weight respectively.

(a) Electrode Implantation. Rats were placed into a Kopf stereotaxic apparatus, the skull exposed, and chronic bipolar twisted stainless steel electrodes implanted into the medial septum nucleus (41) as previously described (10).

(b) Cannula Guide Implantation. Immediately thereafter holes were drilled into the skull over the right hemisphere to accommodate a guide cannula of 20 gage teflon tubing (Small Parts Inc, Miami FL). The tip of the guide was placed on the border of the right SN according to the following coordinates, anterior-posterior [AP] −5.6mm, medial-lateral [ML] 2.25mm, dorsal-ventral [DV] 6.0 mm (41). The cannula guide and electrode were secured to the skull with dental cement and 3 bone screws (0/80 × 1/8 inch). The length of the plastic guide cannula was 1.72 cm and a 1 cm long plastic cuff was slipped over the upper end. A 1.8 cm long stainless steel insect pin was lodged inside the bore of the tubing with the pin head protruding at the upper end. This pin was removed just before injection of γ-vinyl GABA [GVG] (42) with a 27 gage needle extending approximately 0.3 mm beyond the cannula guide into the SN. Correct placement was periodically verified using dye injections.

(c) Jugular Vein Cannulation. Two days prior to experimentation the right external jugular vein was exposed under anesthesia and catheterized with P.E. 10 tubing filled with heparinized (1000 units/ml) normal saline to prevent blockage. It was advanced 2 to 3 cm down the vein toward the heart after insertion. The tubing was tied securely to the vein below and above the point of entry and the free end heat sealed and routed under the skin to exist over the shoulders.

(d) Kindling of Rats. The method employed for rats designated to be made hypoosmotic has been described previously (10). Briefly, after electrode implantation, rats were handled daily for 2 weeks and then were given three daily stimulations through the septal electrodes (400 μA, 1sec, 60Hz). This regimen was maintained for 5 working days each week until the rats were fully kindled (43). Stimulation of the medial septum invariably produced after-discharges in the hippocampus and eventually seizures which were classified as stage 1, 2, 3, 4, or 5 in accordance with established criteria (44, 45). Once five consecutive stage 5 seizures were obtained in an animal, it was rested for 1 week and subsequently retested only once weekly to establish seizure threshold stability.

During these weekly tests, rats were stimulated for 1 second periods with a 60-Hz current of varying intensities, starting with 50 μA and progressing at 30 second intervals with increments of 50 μA until a behavioral seizure was elicited. The current intensity was never taken above 1000 μA. When, on three successive weekly testings, the seizure threshold remained stable (±50μA), an animal was considered ready for experimental purposes. At that time, the weight of each rat

was close to 400 gm. Any kindled rat in which seizure thresholds were variable (in excess of ±50 μA) from week to week, or in which seizure threshold exceeded 1000 μA, was discarded. Only the remaining animals (about 40% of the kindled rats) were used for experimentation. The rats designated to be made hyperosmotic were prepared for seizure threshold testing in a manner identical to that described for those tested in the hypoosmotic state.

(e) Preparation of Normal Rats to Test Only the Effect of Kindling upon GABA Metabolism in the Substantia Nigra. Changes in GABA metabolism were measured in rats in which the stimulating electrodes were implanted into the MS at a 20° angle from the vertical at coordinates AP 0.2, ML −1.8, and DV −4.3 (41). This avoided interference with the GVG cannula guide and GVG infusion. Rats so implanted were divided into several experimental groups. Results from 3 of these groups are reported here. Rats in Group A were not electrically stimulated at any time and received the GVG infusion 90 minutes before being sacrificed. This was the unstimulated control group. Rats in Group B were also naive (unkindled), but received, 30 minutes after the GVG infusion, *a single* electrical stimulus to the medial septum (400 μA, 1 sec., 60 Hz) which usually did not elicit a seizure. These rats also were sacrificed 90 minutes after the initiation of the GVG infusion. They constituted a stimulated control group. Rats in Group C were equivalent to those used for testing the effect of osmotic changes. They were fully kindled (see (d) above), rested for at least one week and sacrificed by decapitation 90 minutes after infusion of GVG into the right SN (46, 47). All animals were injected with 3-mercaptopropionic acid [3MPA] (48, 49) into the jugular vein cannula, two minutes before sacrifice. SN tissue samples were obtained as described below.

(f) Induction of Hypoosmolality. After a constant seizure threshold was established (see d above and Figure 1) rats received 5 units of arginine vasopressin intramuscularly. Their seizure threshold was tested 3 to 3.5 hours later. During subsequent weekly testings, these same animals were first injected with the same amount of arginine vasopressin and, 15 min later, infused ip. with a volume of warm water equal to 10% of their body weight. Again, they were tested for seizure threshold 3 to 3.5 hours later.

(g) Induction of Hyperosmolality. After establishment of a stable seizure threshold, rats were injected subcutaneously with a volume equal to 1.2% body weight of 3M NaCl solution. In order to prevent discomfort, injections were made into the scruff of the neck just behind the shoulder blades. This procedure did not require the use of anesthesia and avoided its long-term effects upon seizure thresholds. Plasma osmolality was elevated within less than 20 minutes by 20 to 35 mOsm. Animals were tested for seizure threshold at times equivalent to those used in the hypoosmotically acclimating rats (f).

(h) Experimental Procedures to Test the Effect of Hyper- and Hypo-osmolality Upon Biochemical Parameters in Tissues. Biochemical analyses were performed on a separate group of rats that were age, weight, sex, and strain matched, had implanted electrodes and when appropriate were stimulated in a similar fashion. However the need to infuse GVG 90 minutes before sacrifice and 3MPA 2 minutes before decapitation precluded the use of the same animals for biochemical analyses and for the measurement of seizure thresholds.

(i) Infusion of GVG and 3MPA. Three hours after initiating hyper- or hypo-osmolality, 0.5 μl of physiological saline solution containing 20 μg of GVG was infused into the right SN at a rate of 0.14 μl/min (47). Unless otherwise indicated the rat was sacrificed 90 minutes later by decapitation in the cold room after a single rapid injection, via the jugular vein catheter of 3MPA (49). The 3MPA was injected as a 2M solution adjusted with NaOH to pH 7.2. Injection of 3MPA (0.2 to 0.4 ml) 2 minutes prior to sacrifice prevented essentially all the post-mortem surge of glutamate to GABA in the brain tissues tested (50, 51).

Plasma Osmolality. At the time of sacrifice a 0.5 ml blood sample was drawn into a heparinized syringe. This sample was centrifuged and the plasma osmolality determined using a Westcor vapor pressure osmometer. The heparin in the syringe contributed insignificantly to the osmolality measurement.

(j) Tissue Sampling and Preparation. The post-mortem surge of glutamate to GABA was eliminated by using 3MPA and the GVG infusion stopped the degradation of GABA in the area of the SN affected. Thus it became unnecessary to work with frozen tissues. Nevertheless we found it convenient to have the brain semi-frozen for sectioning and sampling of SN.

After excision from the decapitated head, the brain was placed in powdered dry ice and then removed to allow it to warm sufficiently to become malleable. The exact technique has been described in detail previously (52) and is modeled after a method described for the rat striatum (53). Using the posterior portion of the Circle of Willis as a landmark, the brain was bisected transversely in the area of the medial geniculate bulge, a point designated as −5.8 to −6.0 mm from the bregma in the Paxino and Watson atlas (41). After brief reimmersion in dry ice powder, a 1mm section was cut in which the more gelatinous SN was clearly visible. The section was placed onto a cold surface and 1 mm diameter punches from the right and left SN were removed

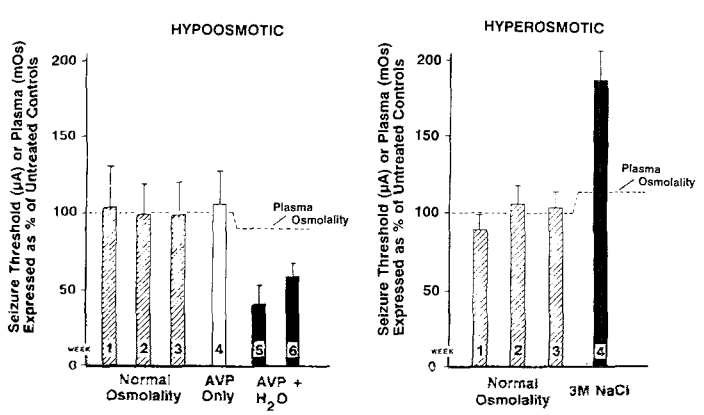

Fig. 1. Effect of Altered Plasma Osmolality upon Seizure Thresholds. AVP = arginine vasopressin. For conditions of testing see text. An increased seizure threshold indicates a decrease in seizure susceptibility.

with separate punch needles. Tissue samples were extruded into a cold glass homogenizer containing 100 μl of ice cold 0.25 M sulfosalicylic acid and 0.06 mM homoserine, the latter as an internal standard for the amino acid assays.

After initial homogenization and deproteinization, an additional 100 μl of deionized water was added before making the final homogenate suspension. All punches were treated in an identical manner. A 40 μl aliquot of each homogenate suspension was removed for protein determination using the bicinchoninic acid [BCA] modification of the Lowry protein assay (54). The remaining suspension was transferred to plastic centrifuge tubes and the denatured proteins precipitated by centrifugation. The supernatant was collected by aspiration and aliquots used for amino acid analyses.

(k) Amino Acid Derivatization, Separation, and Quantitation. Comparative biochemical measurements for amphibian brain have been described (55). Amino acids were separated as O-phthalaldehyde derivatives (56) by HPLC using derivatization, column and elution procedures modified only slightly from those described recently (53, 57). The 150mm steel analytical column packed with C8, 5μ Adsorbosphere (Alltech/Applied Science, Deerfield, IL), was protected by a C8800 guard column packed with the same material and with 2 micron filter elements. The column was used in conjunction with a Spectra Physics SP8700 solvent delivery system, a Kratos model FS 950 fluorescent detector and a Hewlett-Packard 3390A integrator. This integrator was backed up by a Sargent-Welch recorder for quantitation of small and off-scale peaks. The potassium phosphate solution described (58), was replaced with sodium acetate in the derivatization procedure. The HPLC column was kept at 25°C and a 50 μl injection loop was used to apply the sample to the column. Amino acid elution profiles were optimized for the amino acids reported in this paper and values corrected for variations in recovery or derivatization by means of the internal standard. All solutions in these analyses were filtered prior to use. Concentrations of amino acids are expressed as μmoles per g of tissue protein.

(l) In-vitro GABA-T Measurement in SN Tissues. For the measurement of GABA-T as affected by osmolality the method of Cubells et al (59) was used with the following modifications: the strength of the HEPES buffer, pH 8.0, was increased to 70mM and 15 ml of RPI Biosafe II counting fluid was used for the counting of [^{14}C]Succinic semialdehyde.

Four incubation tubes containing 125 μl of incubation mixture were used at each osmolality shown in Figure 2. One tube was inactivated without incubation and was the zero time blank. Two tubes were incubated at 37°C for 30 minutes before inactivation. The contents of the fourth tube was utilized to measure exact osmolality.

The original incubation mixture had an osmolality of 148 mOs. To obtain higher osmolalities either 0.79 M NaCl or 1.0 M mannitol in 70 mM HEPES buffer (pH 8.0) were substituted for a part of the plain HEPES buffer in the original incubation mixture.

RESULTS

(a) Osmolality and Seizure Susceptibility in Kindled rats. A variety of experiments and clinical studies suggest that osmotic conditions influence seizure susceptibility (13, 60). Hypo- and hyper-osmolality in kindled rats had opposite effects (Figure 1). Within the physiological range of blood plasma osmolalities, hypoosmolality lowered seizure thresholds, whereas hyperosmolality elevated seizure thresholds. Both results were statistically significant.

(b) Osmolality Effects on GABA-T. Osmotic changes affect the activity of fully activated GABA-T (Figure 2). This effect is systematic but so minor as to be statistically insignificant within the physiological range of osmolalities used in vivo. The presence or absence of added pyridoxal phosphate in the incubation mixture did not alter the results. Thus it proved unnecessary to make corrections for GABA-T when comparing the rates of GABA accumulation in hyper- and hypo-osmotic rats.

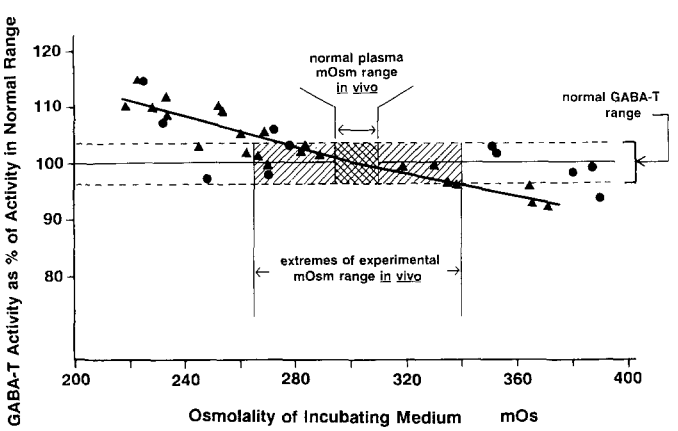

Fig. 2. Effect of Altering Osmolality upon GABA-T Activity in vitro. All tests were run in the presence of optimal amounts of pyridoxal phosphate. Osmolality was altered as described in text using either reagent grade sodium chloride ▲ or mannitol ●. Mannitol was less effective than NaCl in decreasing GABA-T activity especially outside of the osmotic range (265–340 mOs) used for *in vivo* experiments. The normal variability range for GABA-T in rat brain is indicated by the dotted horizontal lines.

(c) Diffusion Measurements of GVG to Test Effectiveness of GABA-T Inhibition in-vivo in SN. Whereas GVG is a most effective suicide inhibitor of GABA-T (61, 62, 42) the rate of diffusion from needle tip into live brain tissues (especially the SN) is finite and has never been determined accurately in the unanesthetized rat. The rate of diffusion and thus the tissue area in which GABA-T is inhibited at different times under our experimental conditions is shown both photographically and graphically in Figure 3. After an initial 10 to 15 minutes, the rate of diffusion into the tissue appeared essentially linear (Figure 3). The methods used for the diffusion measurements follow closely those outlined by Gale (42). Attempts were made to correct for the incomplete inhibition of GABA-T in SN tissues immediately after GVG infusion, an effect that can be seen both in the photographs and area calculations in Figure 3. Since the action of GABA-T in the sections is indicated by the dark (blue) color, a white area indicates GABA-T inhibition. Diffusion during early time periods was not equal in all directions. Interactive image analysis was used (IBAS Kontron Bildanalyse, West Germany), with a magnification factor of about 2000 pixels/mm^2, to calculate the inhibited area as an idealized circle. The method is analogous to sampling and averaging many widths in many directions across the inhibited area from the point of diffusion.

The net outward diffusion rate after 15 minutes was calculated as 0.043 mm/min. It is interesting to note that in experiments with anesthetized rats, injected stereotaxically with GVG, without the use of the plastic canula guide, the initial rate of GVG penetration into the brain tissues was more rapid, but subsequently the linear rate of diffusion was only about 0.017mm/min. Since the infusion of GVG into SN is unavoidably accompanied by some back pressure, it is possible that the minimal space between the plastic needle guide and the injection needle used in experiments with conscious, freely moving, rats, acted as an unintentional reservoir for GVG from which it diffused into the tissues after the first 15 minutes. Making a worst scenario assumption of incomplete inhibition of GABA-T in the right SN for up to 15 minutes after initiating GVG infusion, it can be guestimated that the actual rate of GABA formation in vivo could be up to 16% greater than that shown in Tables I, II, and III. Consequently the true GABA pool turnover time might be a little shorter than indicated in the Tables. However these potential errors are so small that overall results and conclusions are unaffected by them.

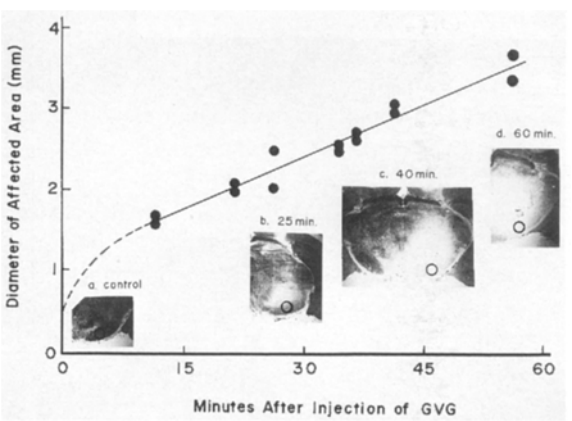

Fig. 3. Temporal Diffusion from Site of Injection of GVG in Brain Tissues. The diameter of the area into which GVG had diffused was calculated as indicated in the text and is plotted here for times after GVG injection. The round circles in the photographs of the substantia nigra sections from hemisected brain indicate the position of the tissue punch that was removed for analysis. See text for details. The minutes indicated above each photograph (a to d) indicate the time that elapsed after the GVG infusion.

(d) Glutamate Decarboxylase Activity in SN. The accuracy of the calculation of in vivo GABA synthesis and turnover are subject to further possible discrepancies. A small pool of glutamate in brain tissue is converted rapidly post-mortem to GABA, thereby increasing the apparent rate of GABA synthesis. To avoid this error, all animals in this study were injected with a single dose of 3MPA as outlined in the methods section (h) (49, 51). This strategy avoided the post-mortem flush from glutamate to GABA. Furthermore, since this post-mortem effect applies equally to the experimental and control sides of the SN and the GABA accumulation rate is calculated by the difference between the two sides, any error introduced should be very small indeed.

(e) The Effect of Hypo- and Hyper-Osmolality Upon GABA Metabolism. GABA synthesis, GABA levels, and GABA turnover were measured in SN in vivo in the unanesthetized rats. The accumulation rate of GABA was calculated by comparing GABA concentrations in the right SN, where GABA degradation was stopped by the infusion of GVG, with the GABA concentrations in the left SN in which the activity of GABA-T remained uninhibited. The assumption was made that in the normal rat unilateral GVG does not alter generalized seizures and that GABA metabolism in right and left SN is equivalent, an assumption that we and other investigators (49, 61) have verified repeatedly.

Results are tabulated in Tables I and II. The level of the GABA pool was decreased slightly but significantly under hypoosmotic conditions (Table I). On the other hand the effects of hyperosmolality (Table II) were quite profound. A relatively small increase in plasma

Table I. GABA Turnover in Rat Substantia Nigra In Vivo as Affected by Hypo-Osmotic Blood Plasma

	Plasma Osmolality mOsm range	GABA pool size in SN μMole/g prot. (±SEM)	GABA "synthesis" μMole/g prot./hr (±SEM)	GABA pool turnover time minutes (±SEM)
Normal rats (n=11)	295–310	109 (±9)	69 (±14)	127 (±25)
Hypo-osmotic rats (n=8)	265–280	82 (±6)	62 (±7)	93 (±15)
p value	p<0.002	p<0.02	ns	ns

Table II. GABA Turnover in Rat Substantia Nigra In Vivo as Affected by Hyper-Osmotic Blood Plasma

	Plasma Osmolality mOsm range	GABA pool size in SN μMole/g prot. (±SEM)	GABA "synthesis" μMole/g prot./hr (±SEM)	GABA pool turnover time minutes (±SEM)
Normal rats (n=10)	295–310	93 (±9)	80 (±14)	80 (±25)
Hyper-osmotic rats (n=7)	330–340	106 (±6)	44 (±7)	210 (±15)
p value	p<0.002	ns	p<0.03	p<0.003

osmolality, during a short time period, produced a small increase in the GABA pool size. This increase has been measured in other brain areas and in whole brain, and becomes significantly larger for longer time periods (55, 10). Even at short time periods, however, the changes in the rate of GABA synthesis and the GABA turnover time in SN tissues of the hyperosmotic rat were highly significant as compared to the control animals.

(f) GABA Turnover and Metabolism in Electrically Stimulated Rats. The two groups of rats, those used for measuring electrophysiological parameters and those used for measuring biochemical parameters in SN, were originally metabolically alike as judged by GABA measurements (Table III). The GABA pool size, the rate of GABA synthesis, and the turnover of the GABA pool remained unchanged in unkindled controls (A) and in rats receiving a single electrical stimulation (B). Even in fully kindled rats (C) the average rate of GABA synthesis and pool size changes were very small, and despite the sizable number of animals tested, of no statistical significance. The kindled rats (C) after one week of rest, were metabolically like the control groups (A and B) as judged by our GABA measurements (Table III). Thus the changes presented in this communication for the SN are the result of osmotic changes and not the result of the kindling procedure used.

[Using an entirely different protocol for kindling, with subsequent stimulation at the time of the study, it is possible to demonstrate an altered GABA metabolism in SN of the kindled rat. The informed investigator who has read our very recent abstract on this subject (63) is cautioned that these latter experimental results cannot and should not be equated or compared with the results in the present paper in which the primary objective was the demonstration of changes in GABA metabolism due to osmotic changes.]

DISCUSSION

It has been shown (Table I and Fig 1) that hypoosmolality is accompanied by a decrease in seizure threshold and a small but significant decrease in the GABA pool size in SN. Hyperosmolality, on the other hand,

Table III. Effect of Kindling on GABA Metabolism in Rat Substantia Nigra

	Plasma Osmolality mOsm range	GABA pool size in SN μMole/g prot. (±SEM)	GABA "synthesis" μMole/g prot./hr (±SEM)	GABA pool turnover time minutes (±SEM)
[A] (n=4) Unkindled NO Stimulation	295–310	93 (±14)	57 (±8)	97 (±5)
[B] (n=3) Unkindled Single Stimulation	295–310	91 (±15)	57 (±11)	97 (±7)
[C] (n=6) FULLY kindled NO stimulation	295–310	107 (±7)	68 (±7)	98 (±10)
p value (A+B vs C)	ns	ns	ns	ns

leads to an increase in seizure threshold and a spectacular increase in the GABA pool size, the rate of GABA synthesis, and GABA turnover (Table II and Figure 1).

The choice of the kindled rat provided a model with a stable seizure threshold, with high relevance to human epilepsy. It is interesting to note that changes in seizure susceptibility under hypo- and hyper-osmotic conditions followed precisely those changes predicted by earlier studies in a variety of species (13, 64, 33). Our observations (10, 65) and those of others (66, 13, 11, 67) suggest that these results are linked firmly to hydration and dehydration but not necessarily to a particular ionic concentration such as Na^+.

The methodology for the measurement of regional GABA pool turnover described here differs from that used by a number of other investigators who favored the use of microwave fixation to rapidly inactivate GABA metabolism (68–71). The discovery that microwave fixation also destroys diffusion barriers for GABA (52) and thus makes regional measurements of GABA unreliable, provided the incentive for the use of 3MPA to avoid the post-mortem flux of glutamate to GABA (49, 50). It should be recognized that GVG as a suicide inhibitor of GABA-T and anticonvulsant is satisfactory for this study but certainly not without shortcomings (61, 72, 73). New and possibly more specific compounds for GABA-T inhibition are being reported (74). Since the effects of osmotic change on GABA metabolism are large (Tables I and II), the conclusions drawn here are unaffected by the slight shortcomings inherent in the methods used (Fig. 2 and 3). As pointed out by others, GABA turnover (unlike GABA content) can be used as a measure of GABAergic function (75, as quoted in 53). The methods we have used take advantage of the fact that unilateral changes in GABA concentration in SN do not modify seizures (33); consequently the rise in GABA over time in the SN injected with GVG is unlikely to have significant feedback effects on local release mechanisms.

As outlined in the introduction, the GABA system and related systems appear closely linked to convulsant and anticonvulsant conditions, to kindled seizures and to epilepsy, with special emphasis on the SN (13, 15, 76, 77, 78). How the GABA system exerts its effect remains controversial (25, 79) but one valid mechanism does not exclude another and there are doubtlessly a number of control points.

The decrease in seizure thresholds (i.e. increase in seizure susceptibility) measured in the hypoosmotic state confirms previous evidence that hypoosmolality is epileptogenic (9). The decrease in GABA pool size in SN suggests that, prior to GVG infusion, GABA release was increased, and this depleted the pool size while the rate of synthesis did not change. Such an interpretation is compatible with the measured increases in excitability, which would be expected to increase cell firing and transmitter release. The 20% pool depletion measured could be sufficient to facilitate seizure spread, in view of the key role of the SN in this process (33). It has been reported and evidence presented by others that when chronic localized infusion of GABA into the cortex of baboons or amygdala-kindled rats is terminated, a rebound effect occurs with increased brain excitability and epileptogenic discharges originating from the earlier infused area. This so-called "GABA withdrawal syn-

drome" has been cited as a new valid model for focal epilepsy (80) and its causes could be similar to those described here for the rat in a hypo-osmotic state (Figure 1, Table I).

The increase in seizure threshold (i.e. a decrease in seizure susceptibility) in hyperosmotic rats as reported here (Figure 1) parallels effects observed in primates. For example, hyperosmolality in man can lead to coma (5). The decreased excitability noted in our rats (Figure 1) may have been associated with a reduction of cell firing rates in SN with an associated major increase in the GABA pool turnover time (Table II). This interpretation would suggest that slower GABA synthesis and turnover in SN of the hyperosmotic rat are a consequence of decreased neuronal activity rather than its cause. Our overall results suggest that GABA metabolism in SN plays an important role in the changes of cerebral excitability associated with hypo- and hyper-osmolality, and that the role of long GABA feedback loops needs to be taken into account when studying metabolic encephalopathies.

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