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The Release of Endogenous GABA and Glutamate from the Cerebral Cortex in the Rat

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Summary. 1. The release of endogenous GABA and glutamate from the cerebral cortex was measured using a cortical cup technique in unanaesthetized freely moving rats and anaesthetized rats by means of a sensitive and specific mass-spectrometric procedure.

2. GABA release was not affected by the presence of the dura mater or by anaesthesia. Glutamate output was reduced by urethane but not by pentobarbital anaesthesia and by the presence of the dura.

3. An isotonic solution containing 50 mM KCl placed epidurally within the cup elicited a significant short-lasting increase in glutamate output, a decrease in GABA output and a short-lasting electrocorticogram (ECoG) activation.

4. When the dura was removed, a high K^+ solution placed on the exposed cerebral cortex elicited a 7–8 fold increase in GABA output accompanied by a marked decrease in glutamate output and by ECoG synchronization. The changes in GABA and glutamate output had parallel time-course and were prevented by the application within the cup of tetrodotoxin (3×10^{-5} M).

5. Amphetamine at the doses of 3.7 and 7.4 μmol · kg^{-1} i.v. increased glutamate output and at the dose of 37 μmol · kg^{-1} i.v. increased GABA output. Both effects were prevented or reduced by haloperidol pretreatment (0.65 μmol · kg^{-1} i.v.).

6. It is concluded that GABA and glutamate released from the cerebral cortex and diffused into an epidural or cortical cup originate at least in part from the brain. The rate of their release is influenced by changes in neuronal activity. The measurement of their rate of release offers a useful tool for the study of the functional role of cortical GABA and glutamate-releasing neurons.

Key words: Neurotransmitter release – GABA – Glutamate – Amphetamine

Introduction

The rate of neurotransmitter output from a brain region is assumed to reflect the activity of the neurons from which the neurotransmitter is released. This assumption has been verified for acetylcholine (Pepeu 1973), 5HT (Aiello-Malberg et al. 1979) and dopamine (Leviel et al. 1979) output.

Several technical reasons, however, have hampered investigations in the release of endogenous GABA and glutamate in vivo. Jasper and Koyama (1969) demonstrated in the cat, using an automatic aminoacid-analyzer, that GABA is released only during periods of slow-wave sleep or after destruction of the midbrain reticular formation. In the same animal species, Iversen et al. (1971) demonstrated by an enzymatic fluorimetric assay that the resting release of endogenous GABA was increased during cortical inhibition produced by electrical stimulation of brain surface or the lateral geniculate nucleus. Clark and Collins (1976) showed in the rat visual cortex, by means of a microdansilation method, an increase in GABA release and a decrease in glutamate release during KCl-induced spreading depression.

However, blood contains GABA (0.37 nmol/ml) (Moroni et al. 1980a) and glutamate (150 nmol/ml of serum) (Liebschutz et al. 1977). Therefore, modifications in cortical blood flow, capillary permeability or blood aminoacid concentration could result in apparent changes in aminoacid release.

In the present experiments the release of endogenous GABA and glutamate from rat cerebral cortex was investigated in vivo by means of a sensitive mass-fragmentographic procedure (Costa et al. 1979; Moroni et al. 1980b). An attempt was made to define the origin of the aminoacids and the effect on GABA and glutamate output of some drugs which modify the functional activity of cortical neurons and the release of other neurotransmitters.

Preliminary accounts of these investigations have been given (Moroni et al. 1980a; Casamenti et al. 1981).

Materials and Methods

Male Wistar rats of 200–250 g body weight were used and were fasted 3 h before the experiments.

The output of endogenous GABA and glutamate was measured using a cortical cup technique either in unanaesthetized freely moving rats with intact dura or in anaesthetized rats.

Freely Moving Rats. A Perspex cylinder (0.25 cm^2 internal surface) was screwed under ketamine anaesthesia (0.40 μmol · kg^{-1}) into the left or right parietal bone according to the method described by Casamenti et al. (1980). Aminoacid release was investigated 3 days after surgery.

Anaesthetized Rats. The rats injected i.p. with urethane (10 mmol · kg^{-1}) were placed in a stereotaxic apparatus; the skull was opened and a Perspex cylinder (0.25 cm^2 internal surface) was placed either on the dura mater or on the exposed fronto-parietal cortex. The rectal temperature was maintained at 37° C by means of an electric pad. In several rats the electrocorticogram (ECoG) was recorded by means of silver ball electrodes applied on the exposed cortex and connected with a Galileo recorder.

In both unanaesthetized and anaesthetized rats 0.4 ml of Ringer solution was placed in the cylinder and substituted every 20 min.

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Mass-Fragmentographic Determination of GABA, Glutamate and Glutamine. The Ringer solution placed in the collecting cylinder had the following composition in mM: NaCl 150, KCl 5.6, $CaCl_2$ 1.6, $NaHCO_3$ 5.9, glucose 5.5. Every 20 min the solution was removed and stored at −70°C until assayed. GABA, glutamate and glutamine were measured using the method described by Bertilsson and Costa (1976), Costa et al. (1979), Moroni et al. (1980b). The method can be summarized as follow: deuterated internal standards of GABA (DL-γ-amino butyric-1.1.2.2.3.3-d_6 acid) 0.02, glutamate (L-glutamic-2-3-3-4-4-d_5 acid) and DL-2-3-3-4-4-d_5 glutamine 0.10 nmoles, dissolved in perchloric acid (0.4 N), were added to the collected samples, and the mixture was loaded into small Dowex columns (AG-50-W-X8 200−400 mesh in acid form). The aminoacids were eluted from the resin with 0.7 ml of NH_4OH 3 N, and the eluate was transferred into Kontes vials and evaporated to dryness under a nitrogen stream. Fifty microliter of 1,1,1,3,3,3 hexafluoroisopropanol and 50 µl of pentafluoropropionic anhydride were added to the dry residue. The vials were sealed and heated for 30 min at 60°C. The reaction mixture was then evaporated and the residue dissolved in 10 µl of ethylacetate. Aliquots of 1−2 µl were injected into a gas-chromatograph-mass-spectrometer LKB 2091 equipped with a multiple ion detector. The separation was made on a 2.5 m × 1 mm i.d. silanized glass column packed with 3% OV 1 on 80−100 Supelcoport (Supelco). The chromatographic conditions were: column temperature 120°C, flash heater 200°C, helium flow 12 cm^3/min. The following ions were recorded with the multiple ion detector: *m/e* 230 for glutamate and glutamine; *m/e* 235 for deuterated glutamate and glutamine and *m/e* 232 and 238 for GABA and deuterated GABA, respectively. The method allows the determination of 10 pmol of the aminoacids. Since the glutamine data are not essential for the goals of the present paper they are not reported under the result section.

Drugs and Chemicals used in this Study. *d*-Amphetamine and *l*-noradrenaline bitartrate, kindly supplied by Recordati, pentobarbital (Nembutal Abbott), ketamine (Ketalar, Parke Davis) and haloperidol (Serenase Lusofarmaco) were used. Deuterated GABA, glutamate and glutamine were obtained from Kor Isotope, Cambridge, Mass. 1,1,1,3,3,3, hexafluoroisopropanol and pentafluoropropionic anhydride were purchased from Pierce, Eurochemie B.V.

Results

Spontaneous GABA and Glutamate Release

The spontaneous output of GABA and glutamate from the cerebral cortex in freely moving and anaesthetized rats is shown in Table 1. The output, measured every 20 min, remained remarkably constant over a period of at least 3 h. GABA output was neither affected by the presence of the dura nor by urethane anaesthesia. Conversely, glutamate output was significantly decreased by urethane but not by pentobarbital anaesthesia and was hampered by the presence of the dura.

In some experiments Ca^{2+} was removed from the Ringer solution applied on the exposed cortex in anaesthetized rats. No changes were observed in the spontaneous release of GABA and glutamate.

Effect of KCl Application on Aminoacid Output and ECoG

After collecting 3 control samples, an equimolar concentration of NaCl was substituted with KCl 50 mM in the Ringer solution filling the collecting cup. When the dura mater was present, the epidural application of KCl caused in both unanaesthetized and anaesthetized rats a long-lasting marked decrease in GABA output associated with a short-lasting increase in glutamate release as shown in Fig. 1. The application of KCl 50 mM also induced a change in the ECoG which was characterized by a 10−15 min period of low voltage high frequency waves followed by a general depression of the electrical activity (Fig. 2).

Conversely, when the dura mater was removed, the application of KCl 50 mM on the cortical surface was followed by an 8 fold increase in GABA release and by a parallel decrease in glutamate release as shown in Fig. 1. The same increase in GABA output was also obtained when Ca^{2+} was omitted in the Ringer solution placed in the collecting cup. The increase in GABA output was accompanied by the appearance of a long-lasting ECoG pattern characterized by low frequency high voltage waves (Fig. 2).

Effect of Tetrodotoxin on KCl-Induced GABA and Glutamate Output

In order to ascertain whether the large increase in GABA output elicited by the application of KCl-enriched Ringer

Table 1. Spontaneous output of GABA and glutamate from rat cerebral cortex

Drug	Dose mmol · kg^{-1} i.p.	Conditions	Output nmol · cm^{-2} · 20 min^{-1} ± SE	
			GABA	Glutamate
None	–	intact dura (5)	0.104 ± 0.008	6.10 ± 0.65[a]
Urethane	10	intact dura (5)	0.072 ± 0.018	4.10 ± 0.40[b]
Urethane	10	removed dura (5)	0.080 ± 0.014	16.4 ± 2.1[c]
Pento-barbital	0.15	intact dura (3)	0.090 ± 0.016	6.3 ± 0.85[d]

Number of animals in parenthesis

The output was measured in 3 consecutive samples for each animal

[a] Versus b $P < 0.05$

[b] Versus c $P < 0.01$

[a] Versus c $P < 0.01$

[b] Versus d $P < 0.05$

[c] Versus d $P < 0.01$

The analysis of variance (two tailed) and the Dunnet *t* test corrected for unequal sample size were used (Winer 1971)

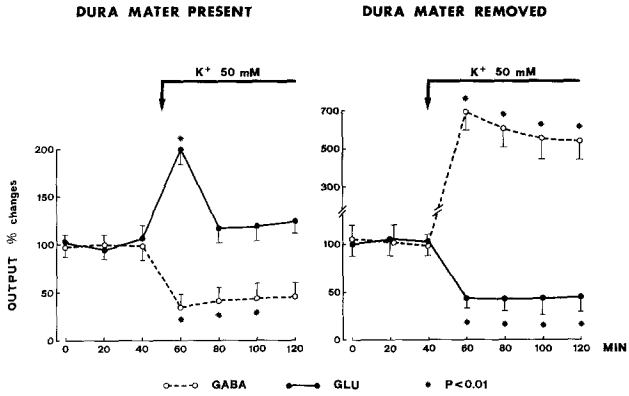

Fig. 1. The effect of locally applied KCl (50 mM) on GABA and glutamate (GLU) output from the cerebral cortex of urethane-anaesthetized rats with and without dura mater. Each point is the mean of at least 5 rats. Vertical bars = standard error of the mean. The difference from basal output is statistically significant with $P < 0.01$ according to the analysis of the variance (two tailed) and the Dunnet *t* test

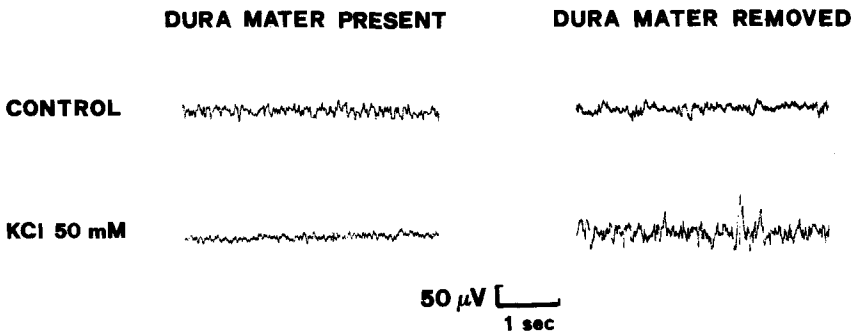

Fig. 2
The effect of KCl (50 mM) on the ECoG in urethane-anaesthetized rats (see text for details)

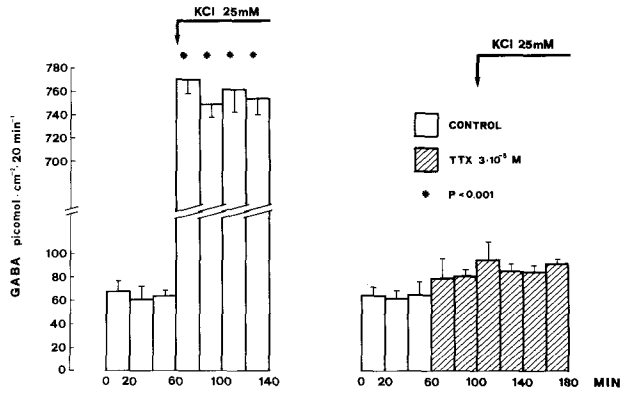

Fig. 3. Antagonism by tetrodotoxin (TTX 3×10^{-5} M) of the KCl (25 mM) induced GABA release from the cerebral cortex in urethane-anaesthetized rats with the dura removed. Each column is the mean value of 5 rats. Vertical bars = standard error of the mean. Statistical analysis as in Fig. 1

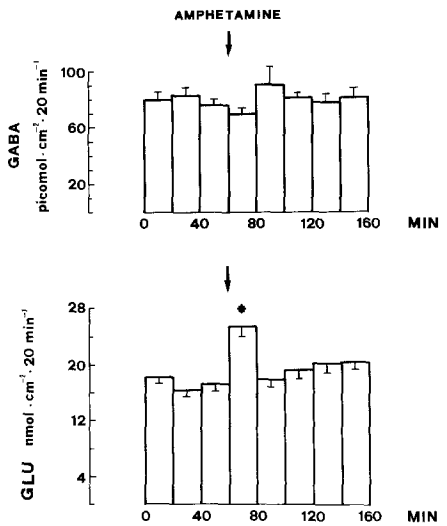

Fig. 4. The effect of amphetamine (7.4 μmol · kg^{-1} i.v.) on GABA and glutamate release from the cerebral cortex of urethane-anaesthetized rats with removed dura mater. Each column is the mean value of 5 rats. Vertical bars = standard error of the mean. * $P < 0.05$. Statistical analysis as in Fig. 1

solution on the cerebral cortex was caused by activation of neuronal firing, tetrodotoxin which blocks the rapid Na^+ influx associated with depolarization (Narahashi et al. 1964) was added to the solution filling the collecting cortical cup.

As shown in Fig. 3, KCl 25 mM, in animals with removed dura mater, elicited approximately a 7 fold increase in GABA

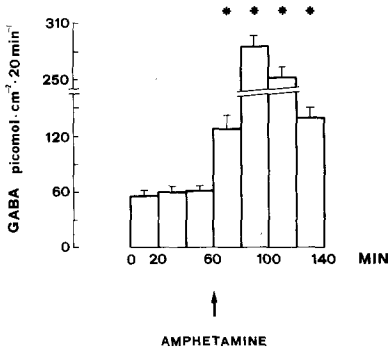

Fig. 5. The effect of amphetamine (37 μmol · kg^{-1} i.v.) on GABA output from the cerebral cortex of urethane-anaesthetized rats with removed dura mater. Each column is the mean value of 5 rats. Vertical bars = standard error of the mean. * $P < 0.01$. Statistical analysis as in Fig. 1

output which was completely prevented by the addition of tetrodotoxin (3×10^{-5} M). The same concentration of tetrodotoxin had no effect on basal GABA output. Moreover, the release of glutamate remained unchanged when tetrodotoxin was added to the Ringer solution containing KCl 25 mM: the mean ± SE of 12 determinations taken from 5 rats for each group was 17.2 ± 2.8 and 16.3 ± 1.5 nmol · cm^{-2} · $20\,min^{-1}$ before and after the addition of tetrodotoxin plus KCl 25 mM, respectively.

Effect of Amphetamine and Haloperidol on GABA and Glutamate Output

Amphetamine was used in order to investigate the relationship between changes in the activity of cortical neurons and GABA and glutamate release. It is known that amphetamine brings about an ECoG activation (Longo and Silvestrini 1957), indicating an increase in the functional activity of the cortical neurons. Amphetamine also stimulates cortical ACh output (Pepeu and Bartolini 1968).

The i.v. injection of 7.4 μmol · kg^{-1} of amphetamine in urethane-anaesthetized rats with removed dura was followed by an increase in glutamate release which occurred only in the first 20 min sample after administration (Fig. 4) and was associated with the presence of low voltage high frequency ECoG waves. The administration of 3.7 μmol · kg^{-1} of amphetamine caused a 60% increase in glutamate output only in 2 out of 5 rats.

On the other hand, the administration of a larger dose of amphetamine (37 μmol · kg^{-1}) was not followed by an increase in glutamate release (data not reported) but by a 2 fold increase in GABA output (Fig. 5). The increased

Table 2. The effects of haloperidol (0.66 μmol · kg^{-1} i.v.) on amphetamine-induced modification of GABA and glutamate output in urethane-anaesthetized animals without dura mater

Pretreatment	Amphetamine μmol · kg^{-1} i.v.	GABA	Glutamate
		% change of basal output	
Saline	7.4	90 ± 6.5	140 ± 10*
Haloperidol	7.4	100 ± 8.2	88 ± 7.9
Saline	37.0	230 ± 20**	85 ± 10
Haloperidol	37.0	152 ± 15*	100 ± 9.8

The aminoacid content of three basal samples was measured and the percent modifications of glutamate efflux were calculated from the first 20 min sample after i.v. amphetamine, while GABA modifications were calculated from the average of the first two 20 min samples after i.v. amphetamine. Haloperidol was injected i.v. 40 min before amphetamine

Each value is the mean ± SE of 4–5 rats

Value significantly different from basal output * $P < 0.05$, ** $P < 0.01$. Statistical analysis as in Fig. 1

GABA release was long-lasting and was still present 1 h after amphetamine administration.

Amphetamine 37 μmol · kg^{-1} i.v. induced an increase in blood pressure from 100 ± 7 to 160 ± 12 mm Hg. The possibility that the changes in glutamate and GABA output might be due to the increase in blood pressure was ruled out by the observation that the infusion of noradrenaline (0.030 μmol · kg^{-1} min^{-1} for 20 min) which caused similar modifications in blood pressure did not affect GABA and glutamate output (data not shown).

Haloperidol antagonizes the behavioural effects (Janssen et al. 1965) and the increase in cortical ACh output (Deffenu et al. 1970) brought about by the administration of amphetamine. Both acute and chronic haloperidol administration did not affect the basal release of GABA and glutamate (Moroni et al. 1980c). However, haloperidol (0.66 μmol · kg^{-1}), as reported in Table 2, completely antagonized the increase in glutamate release following the administration of the small doses of amphetamine and partially reduced the increase in GABA output elicited by the largest dose.

Discussion

Our experiments strongly support the possibility that GABA and glutamate, diffusing into a collecting cup applied epidurally or on the cerebral cortex, originate from the brain. First, the three fold increase in glutamate output when the dura is removed indicates that this aminoacid comes from underneath the dura and does not easily cross this membrane. It also makes it unlikely that glutamate originates mostly from the blood since the removal of the dura does not affect blood aminoacid concentration and vascular permeability. Second, high KCl application in epidural or cortical cups is followed by diverging changes in GABA and glutamate output. It seems unlikely that circulatory or permeability changes induced by KCl could increase the efflux of one aminoacid while decreasing that of the other. Third, GABA and glutamate release are modified by drugs which affect neuronal activity such as tetrodotoxin, amphetamine and urethane while noradrenaline, which increases blood pressure, does not change aminoacid output.

Moroni et al. (1980c) also showed that the intracerebroventricular administration of aminooxyacetic acid, an inhibitor of GABA metabolism, brings about an increase in brain GABA content and output at a time when blood GABA level is not modified.

Neurotransmitter release from nerve endings is a Ca-dependent process (Kelly et al. 1979). However, the removal of Ca^{2+} from the Ringer solution filling the collecting cup reduces neither the spontaneous nor the KCl-enhanced aminoacid output. On the other hand, this procedure might not have been sufficient to decrease in vivo Ca^{2+} concentration at the nerve endings. Clark and Collins (1976) reported that Ca^{2+} removal does not modify the spontaneous GABA output from rat visual cortex in vivo; however, the KCl-evoked release of GABA is abolished when Ca^{2+} in the Ringer solution is replaced by Mg^{2+} with the addition of disodium EDTA. It should be noted that EDTA was not present in the experiments here reported.

The question arises as to where the nerve endings are located from which GABA and glutamate are released. Recent nurochemical findings demonstrate the presence of numerous glutamate-containing neuronal terminals in the outer layer of the cortex while cells containing glutamic acid decarboxylase (GAD; EC 4.1.1.15), a marker for gabaergic neurons, are mainly located in the inner cortical layers (Fonnum 1980). It is therefore possible that the high KCl concentration applied on the dura could only diffuse to a depth sufficient to depolarize glutamate – but not GABA-releasing nerve endings. Whether the decrease in GABA release observed under these conditions is brought about by the increase in glutamate or other neurotransmitter release may be a matter of debate.

When the dura was removed, the application of high K^+ concentration brought about an opposite effect characterized by a large and long-lasting increase in GABA output associated with a decrease in glutamate output. Both changes showed a similar time-course.

Clark and Collins (1976) also observed that the increase in GABA and taurine release from the visual cortex in the rat is usually accompanied by a fall in release of the two excitatory amino acid neurotransmitter candidates glutamate and aspartate. It is possible that GABA released by high K^+ could inhibit glutamate release through an action on presynaptic receptors (Nistri and Constanti 1979). However, muscimol applied into the cup at a concentration up to 8 μM in urethane-anaesthetized rats without dura mater did not decrease glutamate release (Moroni, unpublished results).

The changes in GABA and glutamate output elicited by epidural or cortical application of high K^+ concentration are also associated with modification in ECoG pattern. An activated pattern is recorded when the epidural application stimulates glutamate release and a synchronized pattern when the cortical application enhances GABA output. It is pertinent to mention that Jasper and Koyama (1969) observed an increase in GABA output in the cat during periods of slow wave sleep and that its release was undetectable during ECoG desynchronization. Pepeu et al. (1970) found that during desynchronization the GABA content of cat cerebral cortex is higher than during synchronization. The association between aminoacid release and ECoG changes does not rule out the participation of other putative neurotransmitters in the ECoG modifications and does not mean that the biochemical and electrical phenomena we observed are directly correlated.

From the present experiments it appears that some drugs can modify GABA and glutamate release from the cerebral cortex. Urethane reduces glutamate output, a property not shared by an anaesthetic dose of pentobarbital. It is therefore unlikely that this effect of urethane might be due to a depression of activating influences from the reticular formation.

Amphetamine stimulates glutamate release at low doses and GABA release at higher doses. It is known that amphetamine exerts its central and peripheral actions primarily through the release of dopamine and noradrenaline (Carlsson 1970), which in turn influence other neurotransmitter systems, increasing, for instance, ACh output from the cerebral cortex (Pepeu and Bartolini 1968). The possibility that the biphasic effect of amphetamine on glutamate and GABA output might be due to a dose-dependent differential activation of dopamine and noradrenaline receptors could be envisaged. A biphasic haloperidol-sensitive effect of dopamine agonists on GABA release from the substantia nigra has recently been reported (Van der Heyden et al. 1980). In recent experiments Moroni et al. (1980d) showed that the stimulation of the locus coeruleus, an area from which the cortical noradrenergic fibres originate (Ungerstedt 1971), increased cortical GABA output in the guinea-pig.

In conclusion, it appears that the measurement of GABA and glutamate release from the cerebral cortex in vivo offers a useful tool for studying the functional role of cortical aminoacidergic neurons.

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