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Tonic GABA-ergic modulation of striatal dopamine release studied by in vivo microdialysis in the freely moving rat

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Abstract

$GABA_A$ and $GABA_B$ receptor agonists and antagonists were administered locally in the striatum of intact and kainic acid lesioned rats. (±)-Baclofen, a $GABA_B$ receptor agonist, significantly decreased the level of extracellular dopamine in the striatum of intact rats. (±)-Phaclofen, a $GABA_B$ receptor antagonist, increased the level of extracellular dopamine in the striatum of intact rats and to a lesser extent in the striatum after kainic acid lesion. Pregnanolone (5β-pregnan-3α-ol-20-one), a positive allosteric modulator of the $GABA_A$ receptor, significantly decreased the level of extracellular dopamine in intact rats. (−)-Bicuculline, a $GABA_A$ receptor antagonist, increased the level of extracellular dopamine in the striatum of intact rats, but failed to increase the level of extracellular dopamine after kainic acid lesion. The release of extracellular dopamine, due to infusion of phaclofen or bicuculline, was totally suppressed by tetrodotoxin. These results support a direct influence of GABA on the dopaminergic terminals via presynaptic $GABA_B$ receptors, while the effects via the $GABA_A$ receptor seem to be postsynaptic and mediated by striatal interneurons or the striatonigral feedback loop.

Keywords: Dopamine; Striatum; GABA-ergic drug; Kainic acid

1. Introduction

Dopamine and γ-aminobutyric acid (GABA) are both important neurotransmitters in the striatum. The nerve terminals of the nigrostriatal dopaminergic neurons are located in this part of the basal ganglia. GABA, the main inhibitory neurotransmitter, is contained in the medium-sized spiny neurons of the striatum, which are the GABA-ergic interneurons and the neurons projecting to the substantia nigra via the striatonigral pathway.

Controversial results were published on the effect of GABA-ergic drugs on dopamine release in the striatum. Starr (1978) showed that, in striatal slices, GABA potentiates potassium-stimulated [^{3}H]dopamine release in rat striatum. Giorguieff et al. (1978) found the same stimulatory effect of GABA on non-evoked [^{3}H]dopamine release. However, Reimann et al. (1982) reported that GABA can both inhibit and facilitate dopamine release in the caudate nucleus of the rabbit. In a later report they showed evidence for an inhibitory effect of GABA, baclofen and gabapentin on dopamine release in the rabbit caudate nucleus (Reimann, 1983). Most of these earlier studies were done in vitro which may explain the differences in the results.

The aim of the present work, was to further elaborate on these results by studying the in vivo effects of $GABA_A$ receptor and $GABA_B$ receptor agonists and antagonists on dopamine release and metabolism in rat striatum. To demonstrate the neuronal origin of the released dopamine, the effect of pretreatment with tetrodotoxin was studied in the same model.

The kainic acid-lesioned striatum, where there is destruction of the GABA-ergic striatonigral feedback loop and the interneurons, was used to discriminate between a direct effect of the drugs on dopamine release via the nigrostriatal dopaminergic terminals or an indirect effect.

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2. Materials and methods

2.1. Microdialysis

Male albino Wistar rats weighing about 300 g were used. The animals were given free access to water and food. The rats were anaesthetized with a mixture of ketamine 50 mg/kg and diazepam 5 mg/kg i.p. and placed in a stereotaxic frame. The skull was exposed and an intracerebral guide for CMA/10 probes (CMA/Microdialysis, Stockholm, Sweden) was implanted into the left striatum (L −2.8, A +1.2 and V +3.4; Paxinos and Watson, 1986). The guide was fixed with dental cement and a 3 mm microdialysis probe was immediately inserted through the guide and fixed with a lock screw. The tip of the probe was located at the following coordinates: L −2.8, A +1.2 and V +6.4. The rats were allowed to recover and to get used to the cage. The outer diameter of the tubular dialysis membrane was 0.52 mm and the molecular cut-off point was 20 000 Da. The probes were connected to a microinfusion pump (CMA 100, CMA/Microdialysis, Stockholm, Sweden) and perfused with a modified Ringer's solution (147 mM NaCl, 4 mM KCl, 1.1 mM $CaCl_2$) with a flow rate of 2 μl/min. Dialysates were collected every 20 min in vials containing 80 μl of an antioxidant mixture (0.01 M HCl, 0.1% $Na_2S_2O_5$ and 0.01% Na_2EDTA). The animals were allowed to move freely in the cage.

All the experiments were performed 24 h after implantation of the probes. After six collections, when basal conditions were reached, the drugs were administered for 40 min through the microdialysis probe and dialysates were collected every 20 min for another 3 h.

2.2. Kainic acid lesions

The animals were anaesthetized with the same ketamine/diazepam mixture and placed in the stereotaxic frame equipped with a Hamilton syringe. The lesions were made by injection of 3.0 μl of a 1.0 μg/μl kainic acid (Sigma, St. Louis, MO, USA) in NaCl 0.9% solution pH 6.0, directly into the striatum within 5 min (L −2.8, A +1.2, V +4.9).

All experiments were performed 10 days after the lesion. After the experiments the extension of the lesion was histologically verified. The animals were killed with an overdose of nembutal and their brain removed from the skull. Fixation was performed in a 10% formalin solution. Paraffin sections of 5 μm thickness were stained by hematoxylin-eosin.

2.3. Drugs

All drugs were dissolved in modified Ringer's solution and were administered through the dialysis probe. The following solutions were infused for 40 min at a rate of 2 μl/min: 50 μM (±)-baclofen (Ciba-Geigy, Basel, Switzerland); 100 μM (−)-bicuculline methylchloride (RBI, Natick, MA, USA); 100 μM pregnanolone (5β-pregnan-3α-ol-20-one, Sigma, St. Louis, MO, USA); 2 mM phaclofen (RBI, Natick, MA, USA). The pH of all solutions was verified and adjusted to the pH of the Ringer's solution.

2.4. Measurement of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)

Dopamine, DOPAC and HVA were analyzed by liquid chromatography (LC) with electrochemical detection. The LC system consisted of a Gilson 302 pump (Gilson, Villiers-le-Bel, France) equipped with a 100 μl injection loop (Rheodyne, Cotati, CA, USA). The detector (Chromatofield ELDEC 201, Châteauneuf-les-Martigues, France) was equipped with an electrochemical cell, fitted with a dual glassy-carbon electrode and an Ag/AgCl reference electrode. Separation was performed on a 250 × 4.6 mm reversed phase analytical column (Ultrasphere ODS 5 μm, Beckman, Fullerton, CA, USA). The mobile phase consisted of 98% acetate/citrate buffer containing 0.1 M sodium acetate, 20 mM citric acid, 1 mM 1-octanesulphonic acid, 0.1 mM Na_2EDTA, 1 mM dibutylamine and was adjusted to pH 4.0; 2% of isopropanol was added as the organic modifier. The flow rate was set at 1 ml/min and the detector potential was +700 mV versus the reference electrode. Integration of the chromatograms was performed with a dual channel integration computer program (Integration Pack Kontron, Milan, Italy). The sensitivity of channel 1 for the detection of dopamine was 0.2 nA/V and that of channel 2 for DOPAC and HVA was 2 nA/V.

2.5. Data analysis

Dopamine, DOPAC and HVA levels in the dialysates were expressed as pmol/20 min or pmol/40 μl, without correction for the recovery across the dialysis membrane. In the course of the experiments, levels of dopamine and its metabolites were expressed as percentages of the baseline value, which was the stable value obtained after six collections. The statistical significance of changes in dopamine, DOPAC and HVA levels, compared to the baseline value, was determined with a one-way analysis of variance (ANOVA) for repeated measures and Fisher's protected least significant difference (Fisher PLSD) ($\alpha = 0.05$) was used. The two-tailed unpaired Wilcoxon test was employed for the statistical evaluation of differences between concentrations of the intact and the lesioned striatum ($\alpha = 0.05$).

3. Results

3.1. Basal levels of dopamine. DOPAC and HVA in the striatum of intact and kainic acid-lesioned rats

The basal extracellular levels in pmol/40 μl (= mean levels of the sixth collections, at time 0 min) of dopamine, DOPAC and HVA in the striatum of intact and kainic acid-lesioned freely moving rats are given in Table 1 (mean of ten animals ± S.E.M.).

The basal levels of dopamine in the striatum of the intact and the kainic acid-lesioned rats were significantly different ($P < 0.05$). There was no significant difference for DOPAC and HVA.

To study the effect of the liquid switch in control animals we infused the striatum after six collections with the same modified Ringer's solution for 40 min. No significant change was seen in the concentrations of dopamine, DOPAC and HVA.

3.2. Effect of baclofen on dopamine release and metabolism in the striatum of intact rats

Intrastriatal administration of a 50 μM solution of (±)-baclofen ($GABA_B$ receptor agonist) produced a significant decrease in the levels of dopamine. A maximal decrease was obtained of 51 ± 9% of the basal level after 60 min ($P < 0.001$) ($n = 6$) (Fig. 1).

There was a slight but significant increase in the levels of DOPAC and HVA to, respectively, 144 ± 15% and 146 ± 15% of the basal levels after 160 min ($n = 6$) ($P < 0.05$) (results not shown).

3.3. Effect of phaclofen on dopamine release and metabolism in the striatum of intact rats and of kainic acid-lesioned rats

The $GABA_B$ receptor antagonist, (±)-phaclofen 2 mM, significantly stimulated the release of dopamine in the striatum of intact rats and in the lesioned striatum of kainic acid-treated rats.

Table 1
Basal levels of dopamine, DOPAC and HVA in the striatum of intact and kainic acid-lesioned freely moving rats

	Intact striatum	Lesioned striatum
Dopamine	0.30 ± 0.06	0.06 ± 0.01 [a]
DOPAC	48.3 ± 9.2	32.7 ± 10.5
HVA	20.2 ± 3.7	20.4 ± 3.8

The basal extracellular levels in pmol/40 μl of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum of intact and kainic acid-lesioned rats are given as the mean dialysate concentrations obtained from ten animals ± S.E.M.
[a] The value for the kainic acid-lesioned striatum which is significantly different from the value for intact striatum ($P < 0.05$).

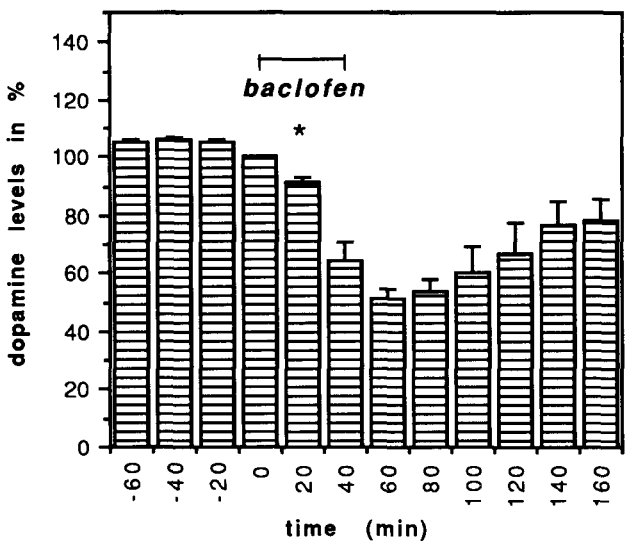


Fig. 1. Effect of intrastriatal administration of 50 μM (±)-baclofen on striatal extracellular dopamine levels in the intact freely moving rat ($n = 6$). Dialysates were collected every 20 min. Baclofen was dissolved in modified Ringer's solution and administered through the dialysis probe from time 0 min to 40 min. Results (means ± S.E.M.) are expressed as percentage of the baseline value. * The first point for which the value was significantly different from the baseline value ($P < 0.05$).

In the intact rats and in the kainic acid-lesioned rats dopamine levels reached, respectively, 287 ± 37% and 179 ± 9% of the basal value after 40 min ($n = 6$) ($P < 0.01$) (Fig. 2).

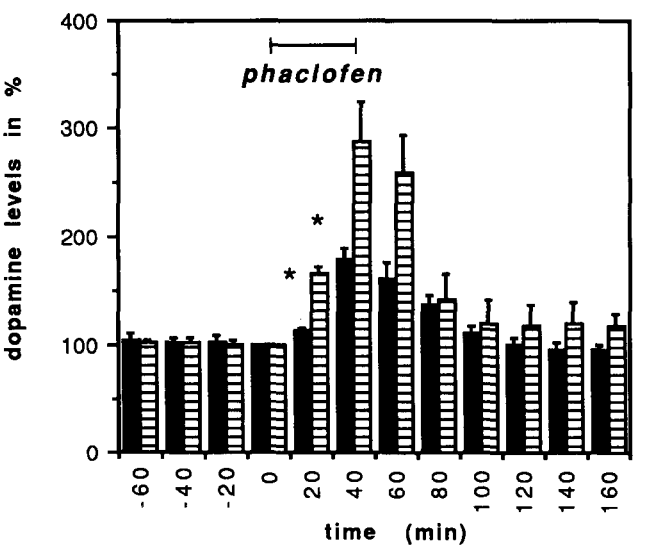


Fig. 2. Effect of intrastriatal administration of 2 mM (±)-phaclofen on striatal extracellular dopamine levels. Phaclofen was administered through the dialysis probe from time 0 min to 40 min. Results (means ± S.E.M.) are expressed as percentage of the baseline value. The effect was compared in intact rats (striped bars) ($n = 6$) and in kainic acid-lesioned rats (black bars) ($n = 6$). * The first point for which the value was significantly different from the baseline value ($P < 0.05$).

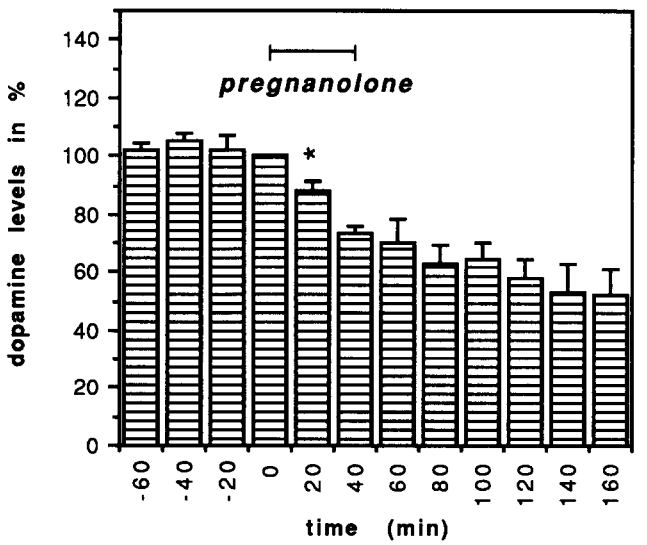


Fig. 3. Effect of intrastriatal infusion of 100 μM pregnanolone (5β-pregnan-3α-ol-20-one) on striatal extracellular dopamine levels in the intact freely moving rat ($n = 6$). Pregnanolone was administered through the dialysis probe from time 0 min to 40 min. Results (means ± S.E.M.) are expressed as percentage of the baseline value. * The first point for which the value was significantly different from the baseline value ($P < 0.05$).

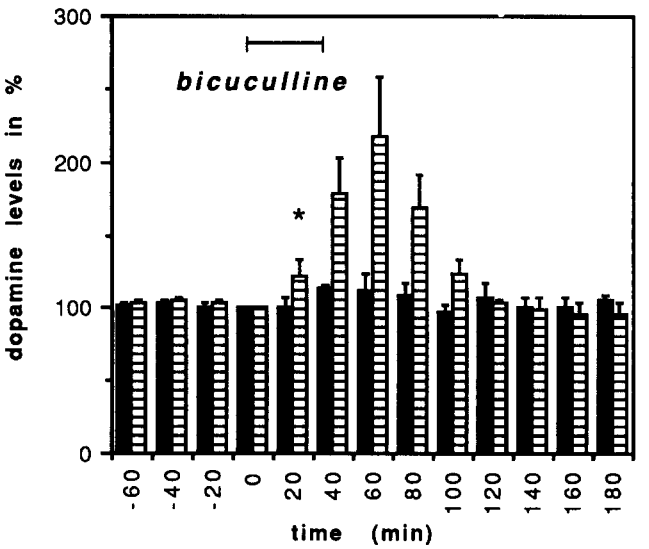


Fig. 4. Effect of intrastriatal administration of 100 μM (−)-bicuculline methylchloride on striatal extracellular dopamine levels. (−)-Bicuculline methylchloride was administered through the dialysis probe from time 0 min to 40 min. Results (means ± S.E.M.) are expressed as percentage of the baseline value. The effect was compared in intact rats (striped bars) ($n = 6$) and in kainic acid-lesioned rats (black bars) ($n = 6$). * The first point for which the value was significantly different from the baseline value ($P < 0.05$).

The effect of phaclofen on the release of dopamine in the intact and that in the kainic acid-lesioned striatum were significantly different ($P < 0.05$). There was no significant effect of the drug on the levels of DOPAC and HVA in the intact and in the kainic acid-lesioned rats.

3.4. Effect of pregnanolone on dopamine release and metabolism in the striatum of intact rats

Intrastriatal administration of pregnanolone (5β-pregnan-3α-ol-20-one), a positive allosteric modulator of the $GABA_A$ receptor, produced a significant decrease in the levels of dopamine. With a 100 μM solution of pregnanolone, dopamine levels reached 52 ± 9% after 160 min ($P < 0.001$) ($n = 6$) (Fig. 3). There was no significant effect of the drug on the level of DOPAC. HVA levels decreased significantly to 77 ± 7% after 60 min ($P < 0.05$) ($n = 6$).

3.5. Effect of bicuculline on dopamine release and metabolism in the striatum of intact rats and of kainic acid-lesioned rats

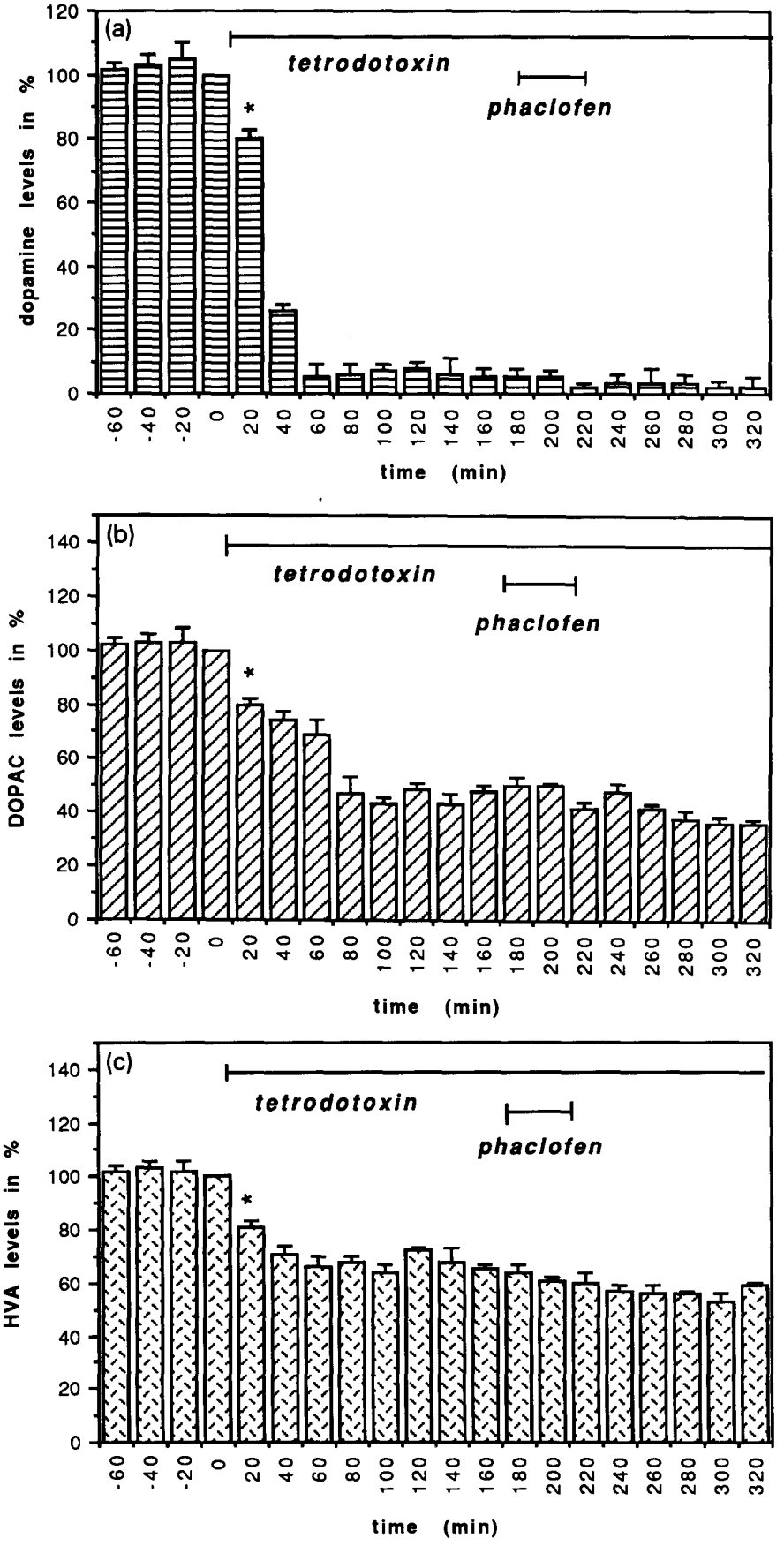
After infusion of 100 μM of the $GABA_A$ receptor antagonist, (−)-bicuculline methylchloride, into the striatum of the intact rats, dopamine levels increased to a maximum of 218 ± 40% of the basal level after 60 min ($P < 0.01$) ($n = 6$) (Fig. 4). The levels returned to baseline after 120 min. There was no significant effect of the drug on levels of DOPAC and HVA.

In the kainic acid-lesioned striatum ($n = 6$) the dopamine levels were 112 ± 11% of the basal level after 60 min and this slight increase was not significant (Fig. 4); neither was there a significant effect on the levels of DOPAC and HVA. There was a significant difference in the effect of 100 μM bicuculline on dopamine release in the intact and in the kainic acid-lesioned striatum ($P < 0.05$).

3.6. Effect of GABA receptor antagonists on the levels of dopamine, DOPAC and HVA in the striatum of intact rats pretreated with tetrodotoxin

To demonstrate the neuronal origin of the dopamine measured in our experiments, we used tetrodotoxin to inhibit the dopamine release induced by the GABA receptor antagonists. Tetrodotoxin 1 μM was infused into the striatum of the intact rats from time 0 min up to 340 min.

Fig. 5. Effect of intrastriatal infusion of 2 mM phaclofen on striatal extracellular levels of dopamine (a), 3,4-dihydroxyphenylacetic acid (DOPAC) (b) and homovanillic acid (HVA) (c) in rats pretreated with tetrodotoxin. From time 0 min 1 μM tetrodotoxin was administered into the striatum for 340 min. At time 180 min 2 mM phaclofen was co-administered into the striatum for 40 min. Results (means ± S.E.M.) are expressed as percentage of baseline value ($n = 4$). * The first point for which the value was significantly different from the baseline value ($P < 0.05$). Phaclofen failed to increase the levels of dopamine, DOPAC and HVA (a, b and c).


(a)
tetrodotoxin
phaclofen
dopamine levels in %
time (min)
(b)
tetrodotoxin
phaclofen
DOPAC levels in %
time (min)
(c)
tetrodotoxin
phaclofen
HVA levels in %
time (min)

In response to 1 μM tetrodotoxin, the dopamine levels decreased to 5 ± 3% of the basal level at time 60 min ($n = 4$) (Fig. 5a). The levels of DOPAC and HVA decreased, respectively, to 42 ± 2% and 65 ± 3% of the basal level at time 80 min (Fig. 5b and c). At time 180 min 2 mM phaclofen was infused simultaneously with tetrodotoxin for 40 min. The drug failed to increase the levels of dopamine, DOPAC and HVA (Fig. 5a–c).

In a similar experiment 100 μM bicuculline also failed to increase the levels of dopamine, DOPAC and HVA (results not shown).

4. Discussion

These results support the hypothesis of a dynamic relationship between dopamine and GABA in the striatum of the freely moving rat.

In a group of animals kainic acid was injected into the striatum to produce an excitotoxic lesion of all the neuronal cell bodies of the striatum, leaving fibres of passage, nerve terminals and glial elements intact (Coyle and Schwarcz, 1976; Herrera-Marschitz and Ungerstedt, 1984). This lesion produced a marked reduction of GABA-ergic and cholinergic markers and striatal atrophy, while leaving dopaminergic neurons relatively unchanged (Schwarcz and Coyle, 1977; Pierce et al., 1992). We found a decrease in extracellular dopamine levels 10 days after injection of kainic acid. It is known that the disruption of the striatonigral GABA-ergic feedback by this lesion produces a short-term increase in dopamine turnover following postsynaptic changes (Naudon et al., 1992). Tissari and Onali (1982) reported that the long-term effect of intrastriatal kainic acid administration is a severe loss of function of striatal dopaminergic terminals. According to Naudon et al. (1992) the metabolic adaptation of these neurons results in a decrease of dopamine turnover and a marked reduction in the monoamine vesicular transporter content, while the neuronal uptake complex content is little affected. We can however not exclude that some of the dopaminergic terminals were destroyed due to the toxic effect of the high dose of kainic acid.

Despite these metabolic changes we saw no significant differences 10 days post-lesion in the levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) compared to those in intact rats, but DOPAC in contrast to HVA had a tendency to be lower in the kainic acid-lesioned striatum. This could be explained by the proliferation of astroglial cells following the degeneration of striatal neurons (Rivett et al., 1983), which could raise catechol-*O*-methyltransferase and monoamine oxidase activities.

After intrastriatal infusion of 50 μM of the selective $GABA_B$ receptor agonist, (±)-baclofen, there was a significant decrease of dopamine release in the rat striatum. These results are in accordance with previous reports of Reid et al. (1990) who showed that intranigral GABA injection decreased striatal dopamine release via a direct action on the nigrostriatal dopaminergic pathway. The GABA-ergic modulation by baclofen is thought to be mediated through $GABA_B$ receptors located on the dopaminergic terminals. Further evidence for this presynaptic localization was provided by the $GABA_B$ receptor-mediated inhibition of tyrosine hydroxylase activity in the striatum of the rat, an effect that was blocked by the $GABA_B$ receptor antagonist, phaclofen (Arias-Montaño et al., 1991). These presynaptic $GABA_B$ receptors are thought to inhibit the release of neurotransmitters through a reduction of the presynaptic Ca^{2+} influx, while the postsynaptic $GABA_B$ receptors may cause hyperpolarization through an increase in K^+ conductance (Bowery, 1989).

(±)-Phaclofen is a phosphonic acid derivative of baclofen and a $GABA_B$ receptor antagonist (Kerr et al., 1987). Phaclofen has a relatively low potency compared to baclofen, which explains the concentration difference for the two drugs. We used 2 mM phaclofen in an experiment with intact and kainic acid-lesioned animals to further investigate the idea of a presynaptic $GABA_B$ receptor on the dopaminergic terminals. Infusion of 2 mM phaclofen into the kainic acid-lesioned striatum could still produce a significant increase in dopamine release, although this increase was not as strong as in the intact rats.

Since the striatal interneurons and striatal efferent neurons are destroyed in the kainic acid-lesioned striatum, the observed increase must be due to an involvement of presynaptic receptors. We suggest that phaclofen blocked the presynaptic $GABA_B$ receptors on the dopaminergic nigrostriatal terminals, resulting in an increase in dopamine release and supporting the hypothesis of a direct tonic inhibition exerted by GABA on the dopaminergic terminals. A second possible explanation could be that phaclofen had suppressed the inhibitory effect exerted by GABA on glutamate release, because the corticostriatal glutamatergic terminals, similar to the nigrostriatal dopaminergic terminals, are not affected by the kainic acid lesion. Additionally, the results of Kilpatrick et al. (1983), suggested the localization of $GABA_B$ binding sites on corticostriatal terminals since there was a 33% reduction in striatal binding after decortication, while there was no apparent reduction in binding after intrastriatal kainate injection only.

The binding of phaclofen to these $GABA_B$ sites could have increased dopamine release indirectly through axo-axonal synaptic glutamatergic stimulation, because a direct facilitatory action of glutamate on dopaminergic terminals has been described (Giorguieff et al., 1977; Clow and Jhamandas, 1988; Leviel et al., 1990; Wang, 1991; Desce et al., 1992).

The difference in response after administration of phaclofen in intact and kainic acid-lesioned rats may reflect an involvement of striatal interneurons or collaterals of striatal efferent neurons in the effect noticed in the intact animals. The attenuation of the response in the lesioned rats can be the result of the loss of indirect glutamatergic stimulation of dopamine release. Indeed, besides the described direct presynaptic glutamatergic modulation of dopamine release, additional evidence for indirect regulation has been reported (Chéramy et al., 1986; Leviel et al., 1990; Desce et al., 1992). Finally, Chesselet (1984) reported that the increase in dopamine release caused by glutamate was not found in kainic acid-lesioned striatum. The difference may also support the idea that there is some axonal loss of afferent neurons as a result of the kainate injection.

The study of the $GABA_A$ receptor is complex due to the existence of its different allosteric binding sites (Siegarth, 1992). Activation of the GABA binding site triggers a conformational change of the receptor and increases the Cl^- conductance of the postsynaptic membrane, resulting in hyperpolarization and reduced membrane excitability (Möhler, 1992). The positive allosteric modulation of certain steroids at the steroid binding site was found to prolong the membrane Cl^- conductance and thus to potentiate the inhibitory effect of the GABA present (Deutsch et al., 1992).

Intrastriatal administration of 100 μM pregnanolone (5β-pregnan-3α-ol-20-one) significantly decreased the level of extracellular dopamine in the striatum of the intact rats. Infusion of allopregnanolone (5α-pregnan-3α-ol-20-one) was without effect on dopamine release (not shown). An anxiolytic effect, mediated by a potent and stereospecific modulation of the $GABA_A$ receptor, was demonstrated for both steroids (Bitran et al., 1991). Our results, obtained with microdialysis, support the idea that GABA controls the basal striatal dopamine release and that pregnanolone enhances the inhibitory effect exerted by GABA on the dopaminergic terminals through modulation of the $GABA_A$ receptor.

Zetterström and Fillenz (1990) found that, after local administration of 10 μM of the benzodiazepine flurazepam, there was a clear reduction in extracellular dopamine levels in the nucleus accumbens, while there was no effect on striatal dopamine levels. Thus flurazepam can act as a positive allosteric modulator at the benzodiazepine binding site of the $GABA_A$ receptor complex. The inability to depress striatal dopamine release could be due to the lower density of benzodiazepine binding sites in the striatum compared to the nucleus accumbens (Zetterström and Fillenz, 1990).

The $GABA_A$ antagonist, bicuculline, acts at the GABA binding site and can competitively antagonize the inhibition of the target neuron (Sieghart, 1992). This was reflected in our results: intrastriatal infusion of 100 μM (−)-bicuculline methylchloride significantly increased dopamine release in the striatum of the intact rats. No effect was seen on dopamine metabolism during the whole experiment. Leviel et al. (1990) observed the same effect with 100 μM bicuculline in an in vivo experiment using the push-pull technique. They reported an increase of $\pm$140% in dopamine release during the first 40 min of application. This is lower than the increase seen in our experiments, but this difference could be due to the direct contact between perfusion fluid and tissue in the push-pull technique or to the use of a racemate of bicuculline.

The increase in dopamine release after infusion of bicuculline was totally lost in the kainic acid-lesioned striatum. So, in contrast to the effect of the $GABA_B$ receptor antagonist, phaclofen, the effect of the $GABA_A$ receptor antagonist seems to be totally dependent on the existence of the striatal interneurons and the striatonigral GABA-ergic feedback loop. This suggests that the $GABA_A$ receptors in rat striatum are located postsynaptically on the GABA-ergic or cholinergic interneurons, or play a modulator role as receptors on the soma or collaterals of the GABA-ergic feedback loop to the substantia nigra.

Although there was a significant effect of all drugs used on dopamine release, there was mostly no significant effect on the levels of DOPAC. Imperato and Di Chiara (1988) reported similar findings for DOPAC after local infusion of dopamine D_1 and D_2 receptor agonists and antagonists. They explained that the area of the striatum affected by local drug application was restricted to the immediate surroundings of the dialysis membrane, so that local changes in metabolite production cannot affect the overall output of dopamine metabolism. Zetterström et al. (1988) proved that a major part of the dopamine metabolite, DOPAC, is derived from an intraneuronal pool of newly synthesized dopamine which has not been recently released, so this mechanism would also explain the poor correlation between changes in the levels of dopamine and DOPAC in perfusates following pharmacological manipulations.

Infusion of 1 μM tetrodotoxin significantly decreased the extracellular levels of dopamine, DOPAC and HVA. Those results were comparable with the findings of Drew et al. (1989). More interesting is the fact that tetrodotoxin totally blocked the dopamine release induced by both the $GABA_A$ and the $GABA_B$ receptor antagonists, which supports its neuronal and vesicular origin. Tetrodotoxin infusion is an established technique to manipulate neuronal activity, because this neurotoxin blocks the voltage-dependent Na^+ channels; tetrodotoxin sensitivity implies that the neurotransmitter is derived directly from neuronal activity (Westerink et al., 1987). In in vivo experiments, this

tool can be used further to discriminate between vesicular (tetrodotoxin-dependent) and carrier-mediated (tetrodotoxin-independent) neuronal release. In in vitro experiments, tetrodotoxin is employed to distinguish direct (tetrodotoxin-resistant), i.e. mediated by presynaptic receptors, from indirect (tetrodotoxin-sensitive), i.e. involving local circuits, regulation (Desce et al., 1992).

In conclusion, our results demonstrate a tonic inhibition exerted by GABA on dopamine release in rat striatum. The dopaminergic terminals seem to be under the control of GABA via presynaptic $GABA_B$ receptors localized on nigrostriatal and corticostriatal nerve terminals. The effects via the $GABA_A$ receptor seem to be postsynaptic and dependent on an unimpaired striatonigral GABA-ergic feedback loop or striatal interneurons.

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