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ORIGINAL ARTICLE

# β-alanine elevates dopamine levels in the rat nucleus accumbens: antagonism by strychnine

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**Abstract** Glycine receptors (GlyRs) in the nucleus accumbens (nAc) have recently been suggested to be involved in the reinforcing and dopamine-elevating properties of ethanol via a neuronal circuitry involving the VTA. Apart from ethanol, both glycine and taurine have the ability to modulate dopamine output via GlyRs in the same brain region. In the present study, we wanted to explore whether yet another endogenous ligand for the GlyR, β-alanine, had similar effects. To this end, we monitored dopamine in the nAc by means of in vivo microdialysis and found that local perfusion of β-alanine increased dopamine output. In line with previous observations investigating ethanol, glycine and taurine, the competitive GlyR antagonist strychnine completely blocked the dopamine elevation. The present results suggest that β-alanine has the ability to modulate dopamine levels in the nAc via strychnine-sensitive GlyRs, and are consistent with previous studies suggesting the importance of this receptor for modulating dopamine output.

**Keywords** Nucleus accumbens · Dopamine · Glycine receptor · Rat

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## Introduction

The inhibitory neurotransmitter glycine has two major functions in the brain where one is as an agonist at the glycine receptor (GlyR) and the other is to facilitate glutamatergic transmission by interacting with the glycine site of the NMDA receptor. Apart from glycine, other endogenous aminoacids such as taurine and β-alanine, to mention a few, are known to have affinity for the strychnine-sensitive GlyR (Pan and Slaughter 1995; Breitinger and Becker 2002). However, depending on which subunit composition of the receptor that is studied, at what age, and in which brain region the study is taking place, there appears to be differences between the properties of the ligands (Pan and Slaughter 1995; Rajendra et al. 1995).

GlyRs in the nucleus accumbens (nAc) have recently been shown to be of importance for regulating basal as well as ethanol-, taurine- and acamprosate-induced dopamine release in the same brain region (Molander and Söderpalm 2005a, b; Ericson et al. 2006; Chau et al. 2007). This receptor, located in the nAc, has also been shown to be involved in anticipation of ethanol (Li et al. 2008), strengthening the suggested importance of this receptor in mediating reinforcing properties of drugs of abuse.

Glycine, taurine and β-alanine are all present in the nAc, which means that they alone, together or in place of each other depending on environmental circumstances, could participate in regulating dopaminergic output in this region. Indeed, both glycine and taurine have been shown to increase dopamine levels by interacting with the strychnine-sensitive GlyR, as demonstrated by the discovery that pre-treatment with the competitive GlyR antagonist prevented this elevation (Molander and Söderpalm 2005b; Ericson et al. 2006).

The present study aimed to investigate whether β-alanine administered by reversed in vivo microdialysis in the nAc could influence dopamine output in the same brain region and, if so, whether this effect was mediated via GlyRs.

## Methods

### Animals

A total of 87 male Wistar rats (Beekay, Stockholm, Sweden) weighing 270–350 g were housed five per cage (55 × 35 × 20) at constant room temperature (22°C) and humidity (65%). The animals were kept under regular light–dark conditions (lights on at 7:00 am and off at 7:00 pm) and had free access to "rat and mouse standard feed" (Harlan Teklad, UK) and tap water. In all experiments drug naive animals were used. Animals were allowed to adapt for 1 week to the novel environment before any experiments were performed. This study was approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden (337/06).

### Drugs

The amino acid β-alanine (Fisher Scientific, Sweden) and the competitive GlyR antagonist strychnine (strychnine HCl, Sigma-Aldrich, Sweden), were dissolved in Ringer solution and administered by reversed microdialysis. The content of the Ringer solution was (in mmol/l): 140 NaCl, 1.2 $CaCl_2$, 3.0 KCl, and 1.0 $MgCl_2$.

### Microdialysis technique

Brain microdialysis experiments were performed in awake and freely moving animals as previously described (Lidö et al. 2009). Briefly, rats were anaesthetized by isoflurane, mounted into a stereotaxic instrument (David Kopf Instruments, AgnTho's AB, Sweden) and put on a heating pad to prevent hypothermia during the surgery. Holes were drilled for the placement of two anchoring screws and an I-shaped dialysis probe custom made in the laboratory. The dialysis probe was lowered into the nAc (A/P +1.85, M/L −1.4 mm relative to bregma, D/V −7.8 mm relative to dura; Paxinos and Watson 2007). The dialysis probes were placed in the core-shell borderline region (suggesting that sampling was done in both the core and the shell of the nAc) and the probes and the anchoring screws were fixed to the skull with Harvard cement (DAB Dental AB, Sweden). After surgery, the rats were allowed to recover for 2 days before the dialysis experiments were initiated. On the experimental day, the sealed inlet and outlet of the probes were cut open and connected to a microperfusion pump (U-864 Syringe Pump, AgnTho's, Sweden) via a swivel allowing the animal to move around freely. The probe was perfused with Ringer solution at a rate of 2 µl/min, dialysate samples (40 µl) were collected every 20 min and were analyzed immediately. The probes were perfused with Ringer solution for 1 h in order to obtain a balanced fluid exchange before baseline sampling began. The length of the active membrane of the dialysis probe was 2 mm and the typical in vitro recovery of dopamine was 5–10%. Animals were sacrificed directly after the experiment, brains were removed and probe placements were verified using a vibroslicer (Campden Instruments Ltd, Leicester, UK; Fig. 1).

### Biochemical assay and experimental procedure

To determine the concentration of dopamine in the dialysate a high-pressure liquid chromatography system with electrochemical detection was used as previously described (Lidö et al. 2009). An external standard containing 2.64 fmol/µl of dopamine was used in order to identify the dopamine peak. When at least three consecutive stable values of dopamine were obtained (±5%) the first drug was introduced. These samples of baseline dopamine were considered 100% and all of the following samples were

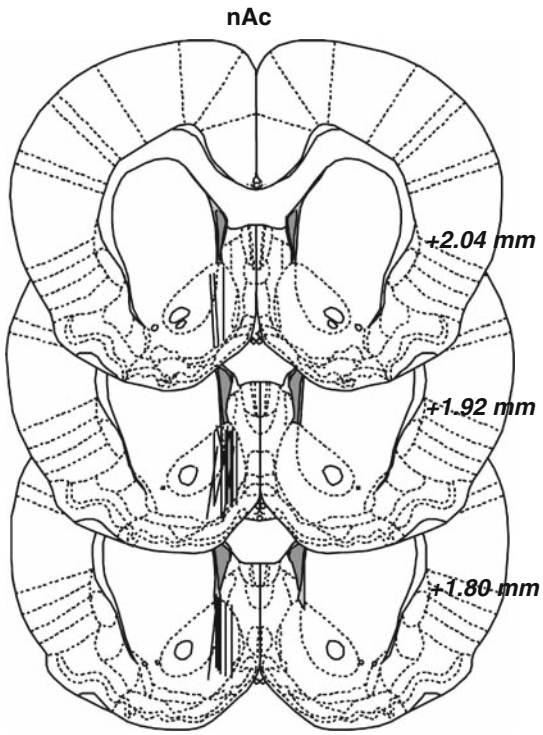


**Fig. 1** Coronal sections of the rat brain indicating the placements of the microdialysis probes (*black lines*) in the nucleus accumbens, only correctly placed probes were included. *Numbers besides each plate* represent distance from bregma

calculated as % change from baseline. In the first set of experiments, animals received either Ringer or β-alanine (0.1, 1 or 10 mM) via reversed dialysis in the nAc. After the second set of rats had established a stable baseline the competitive GlyR antagonist strychnine (2 or 20 μM) was perfused in the nAc alone for 3 h or 3 h with the addition of β-alanine (1 or 10 mM) after 40 min.

### Statistical analysis

Data were statistically evaluated using a two-way ANOVA with repeated measures (treatment group × time) followed by Fisher's protected least significant difference test (PLSD). A probability value ($p$) less than 0.05 was considered statistically significant. All values are expressed as means ± SEM.

## Results

In the first set of experiments, different concentrations of β-alanine were administered into the nAc of the rats by reversed microdialysis. Statistical evaluation using ANOVA with repeated measures revealed that the treatment significantly elevated the extracellular dopamine levels in the same brain region, group effect $F(3, 35) = 5.94$, $p = 0.002$, time effect $F(8, 280) = 15.08$, $p < 0.001$ and interaction effect $F(24, 280) = 4.47$, $p < 0.001$ and the post hoc test demonstrated that all concentrations elevated dopamine output when compared with Ringer perfusion (Ringer vs. β-alanine 0.1 mM, $p = 0.038$, Ringer vs. β-alanine 1 mM, $p = 0.019$, Ringer vs. β-alanine 10 mM, $p < 0.001$; Fig. 2).

In the second set of experiments, where the aim was to investigate whether the β-alanine-induced elevation of dopamine was mediated via GlyRs, we pre-treated animals with the competitive GlyR antagonist strychnine (2 or 20 μM) before perfusion of β-alanine (1 or 10 mM) was initiated. Administration of the lower concentrations of both drugs (2 μM strychnine and 1 mM β-alanine) resulted in no significant alteration of dopamine levels in the nAc when compared with Ringer administration [ANOVA; group effect $F(3, 33) = 9.76$, $p < 0.001$, time effect $F(8, 264) = 2.59$, $p = 0.001$ and interaction effect $F(24, 264) = 3.12$, $p < 0.001$, Fisher's post hoc; β-alanine vs. β-alanine + strychnine $p < 0.001$, Ringer vs. β-alanine + strychnine $p = 0.415$; Fig. 3a]. Pre- and co-treatment of the higher concentrations of strychnine (20 μM) and β-alanine (10 mM) also failed to alter the extracellular levels of dopamine in the nAc [ANOVA; group effect $F(3, 32) = 7.21$, $p < 0.001$, time effect $F(8, 256) = 0.32$, $p = 0.959$ and interaction effect $F(24, 256) = 4.79$, $p < 0.001$, Fisher's post hoc; β-alanine vs. β-alanine + strychnine $p = 0.004$, Ringer vs. β-alanine + strychnine $p = 0.674$; Fig. 3b].

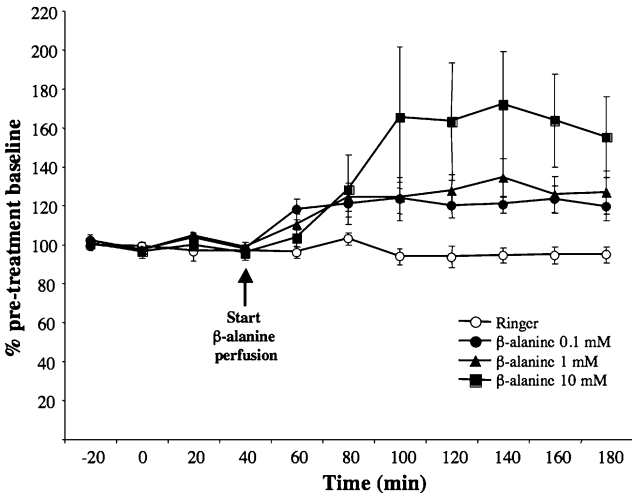


**Fig. 2** Effect of β-alanine (0.1, 1 or 10 mM) or Ringer perfused in the nucleus accumbens on extracellular accumbal dopamine levels, as measured by in vivo microdialysis in freely moving Wistar rats. For statistics, see "Results". Data is presented as means ± SEM; $n$ = 7–14 [Ringer (7), β-alanine 0.1 (8), β-alanine 1 (9), β-alanine 10 (14)]. β-alanine administration was initiated as indicated by the *arrow*

## Discussion

In the present study, perfusion of β-alanine (0.1, 1 and 10 mM) into the nAc was found to increase extracellular dopamine levels in the same brain region. This finding demonstrates that the β-alanine has the same ability to influence accumbal dopamine output as previously shown both with local (nAc) glycine (100 μM and 1 mM) and taurine (10 and 100 mM) administration in freely moving Wistar rats (Molander and Söderpalm 2005a; Ericson et al. 2006). In addition, the present results are also in line with previous studies since pre-treatment with the competitive GlyR antagonist strychnine antagonized the β-alanine-induced elevation of dopamine in the nAc, indicating that the effect was mediated via the strychnine-sensitive GlyR. Whether the elevation of dopamine after β-alanine perfusion is a local mechanism limited to the nAc was not tested in this set of experiments, but as previously demonstrated with both ethanol and taurine it is likely that a neuronal circuitry is activated by β-alanine, disinhibiting GABAergic neurons projecting to the VTA (Ericson et al. 2003, 2006). This disinhibition of GABA release into the VTA results in increased amounts of acetylcholine output, which leads to elevated levels of dopamine in the nAc after activation of ventral tegmental nicotinic acetylcholine receptors in the anterior VTA (Ericson et al. 2003; Larsson et al. 2005; Ericson et al. 2008a).

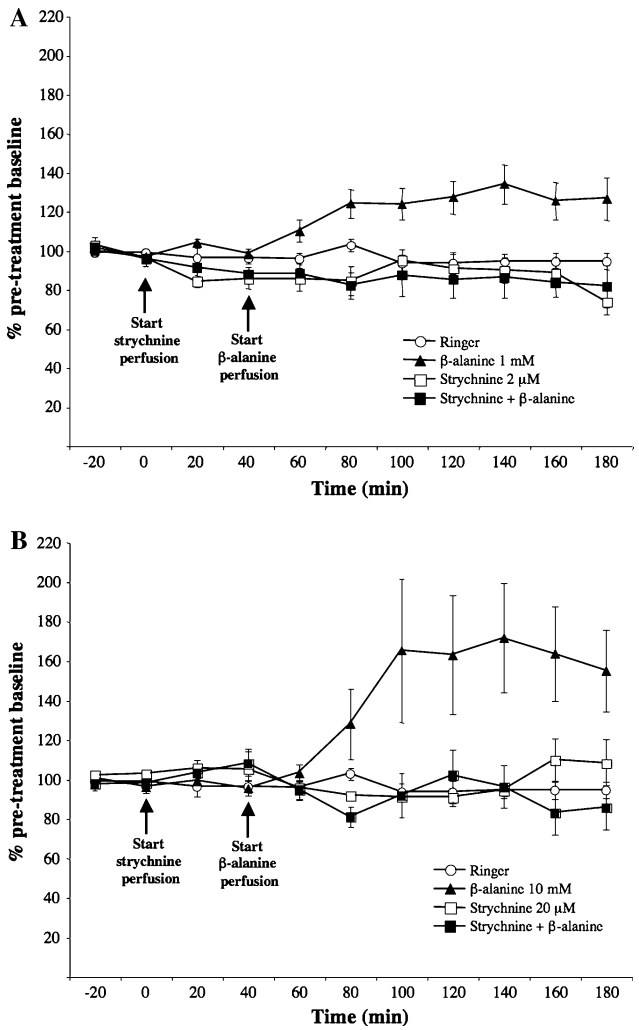


**Fig. 3** Effect of **a** Ringer, *β*-alanine (1 mM), strychnine (2 μM) or the combination of strychnine and *β*-alanine or **b** Ringer, *β*-alanine (10 mM), strychnine (20 μM) or the combination of strychnine and *β*-alanine perfused in the nucleus accumbens on extracellular dopamine levels, as measured by in vivo microdialysis in freely moving rats. For statistics, see "Results". Data is presented as means ± SEM; $n = 7$–14 [Ringer (7), strychnine 2 (8), strychnine 20 (8), *β*-alanine 1 (9), *β*-alanine 10 (14), *β*-alanine 1 + strychnine 2 (9), *β*-alanine 10 + strychnine 20 (8)]. Drug administration was initiated as indicated by the *arrows*

Even though this study only establishes that local perfusion of *β*-alanine in the nAc, in line with glycine and taurine, elevates dopamine levels in the same brain region, it is tempting to speculate upon the physiological function of these three amino acids in relation to dopamine output in the nAc. The subunit composition of the receptor appears to be crucial for the affinity of the different ligands (Pan and Slaughter 1995; Breitinger and Becker 2002). Studies in *Xenopus* oocytes have, for example, shown that GlyRs constituting of α1 subunits are sensitive to *β*-alanine and taurine whereas GlyRs with α2 or α3 subunits are sensitive to *β*-alanine but not taurine (Betz 1991; Malosio et al. 1991). However, taurine has been forwarded as the primary ligand for activation of the embryonal and early postnatal form of the GlyR-containing α2 subunits (Kirsch 2006) indicating that the subunit may alter its properties upon development. Furthermore, mutations of the extracellular domain of the α1 GlyR subunit alters not only the affinity of the different ligands but also the function of the GlyR (Rajendra et al. 1995; Schmieden et al. 1999). Thus, it is possible that glycine, *β*-alanine and taurine all act as ligands for the receptor, and that the receptor alters subunit constellation in order to maintain a balanced system.

Small but consistent amounts of glycine are present in the nAc both in order to facilitate glutamatergic neurotransmission (Reynolds and Miller 1990) and perhaps also to maintain a dopaminergic tone in the nAc. In line with this hypothesis Molander and Söderpalm (2005a) demonstrated that increasing concentrations of strychnine (2–200 μM) decreased dopamine levels in the nAc accordingly suggesting that GlyRs are tonically regulating dopamine output. In the previous study, 20 μM strychnine produced a significant decrease in dopamine output whereas in the present study no such decrease could be detected. In our experience, this particular concentration will produce a small effect in some animals and none in others, which is why we believe we have a discrepancy between the two studies. We hypothesize that under normal conditions glycine, perhaps in combination with *β*-alanine, is responsible for maintaining the dopamine output in the nAc. Taurine, an aminoacid that has often been assigned osmoregulatory properties (Olive 2002), is released in the nAc after ethanol exposure in vivo (DeWitte et al. 1994; Quertemont et al. 2000, 2002). The ethanol-induced increase in extracellular levels of taurine will disrupt the normal milieu and cause an elevation of dopamine output in the nAc by inducing disinhibition of acetylcholine release into the VTA. Indeed, preliminary data confirms that if ethanol is prevented from increasing extracellular taurine no ethanol-induced dopamine elevation will be observed (Ericson et al. 2008b).

*β*-alanine has also been demonstrated to inhibit GABA transport, since the GABA transporter 3 demonstrated higher affinity for *β*-alanine than for GABA (Jurskey and Nelson 1999). This mechanism has indirectly been associated with schizophrenia (Schleimer et al. 2004) as well as in relieving parkinsonian tremor (Ishiwari et al. 2004). In the present study, we cannot exclude that *β*-alanine exerts its dopamine-elevating properties indirectly by elevating extracellular levels of GABA instead of acting as an agonist on the GlyR. However, this appears unlikely considering that GABA has a rather low affinity for the GlyR and would thus require very high amounts in order to mediate a signal via the GlyR.

Taken together, the present study gives further support for the importance of GlyRs in relation to dopamine output in the nAc. This inhibitory receptor and its ligands appears to be involved both in the mechanism underlying ethanol reinforcement (Molander et al. 2005, 2007) as well as in drug anticipation (Li et al. 2008). Even though the receptor needs to be further investigated both in relation to normal and drug-induced function the potential importance for mesolimbic dopamine output appears to be great. The functional importance of β-alanine as a ligand for the GlyRs needs to be further explored in order to understand how different ligands interact in mediating GlyR function.

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