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Chronic inositol increases striatal D_2 receptors but does not modify dexamphetamine-induced motor behavior Relevance to obsessive–compulsive disorder

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Abstract

A large body of evidence suggests that the neuropathology of obsessive–compulsive disorder (OCD) lies in the complex neurotransmitter network of the cortico-striatal-thalamo-cortical (CSTC) circuit, where dopamine (DA), serotonin (5HT), glutamate (Glu), and gamma-amino butyric acid (GABA) dysfunction have been implicated in the disorder. Chronic inositol has been found to be effective in specific disorders that respond to selective serotonin reuptake inhibitors (SSRIs), including OCD, panic, and depression. This selective mechanism of action is obscure. Since nigro-striatal DA tracts are subject to $5HT_2$ heteroreceptor regulation, one possible mechanism of inositol in OCD may involve its effects on inositol-dependent receptors, especially the $5HT_2$ receptor, and a resulting effect on DA pathways in the striatum. In order to investigate this possible interaction, we exposed guinea pigs to oral inositol (1.2 g/kg) for 12 weeks. Subsequently, effects on locomotor behavior (LB) and stereotype behavior (SB), together with possible changes to striatal $5HT_2$ and D_2 receptor function, were determined. In addition, the effects of chronic inositol on dexamphetamine (DEX)-induced motor behavior were evaluated. Acute DEX (3 mg/kg, ip) induced a significant increase in both SB and LB, while chronic inositol alone did not modify LA or SB. The behavioral response to DEX was also not modified by chronic inositol pretreatment. However, chronic inositol induced a significant increase in striatal D_2 receptor density (B_{max}) with a slight, albeit insignificant, increase in $5HT_2$ receptor density. This suggests that D_2 receptor upregulation may play an important role in the behavioral effects of inositol although the role of the $5HT_2$ receptor in this response is questionable.

Keywords: Inositol; D_2 receptor; $5HT_2$ receptor; Locomotor behavior; Stereotype behavior; OCD; Guinea pig

1. Introduction

Inositol is a key metabolic precursor in the phosphatidylinositol (PI) second messenger cycle. Inositol has also demonstrated clinical efficacy in a variety of neuropsychiatric illnesses, including obsessive–compulsive disorder (OCD) (Fux et al., 1996), panic (Benjamin et al., 1995), and depression (Levine et al., 1995a). This therapeutic response appears to be specific for neuropsychiatric illnesses that respond to selective serotonin reuptake inhibitors (SSRIs), while it is ineffective in disorders such as Alzheimer's disease (Barak et al., 1996), autism, and schizophrenia (Levine, 1997). Moreover, inositol appears to worsen attention-deficit hyperactivity disorder (ADHD) (Levine et al., 1995b). Its exact mechanism of action in these disorders is speculative, other than the pure assumption that it replenishes the membrane PI pool. PI synthetase is the rate-limiting step in the synthesis of PI and is considered a saturated system. Therefore, unless an overt depletion of brain PI exists, it is not expected that precursor supplementation would be of any marked value (Levine, 1997). Nevertheless, studies have found that exogenous inositol does have marked effects on behavior (Cohen et al., 1997; Kofman et al., 1993, 1998) and on cell function, including regulating the activity of phospholipase C (PLC) (Batty and Downes, 1995) and preventing $5HT_2$ receptor desensitization (Rahman and Neuman, 1993).

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Considerable evidence points to the involvement of the striatum, especially the head of the caudate nucleus, and the orbito-frontal and cingulate cortex, in the neuropathology of OCD (Insel, 1992). In all these areas, an increased functional activity has been observed during OCD (Insel, 1992). Furthermore, this response is suppressed by SSRI treatment with an associated improvement in obsessive–compulsive (OC) symptoms (Baxter et al., 1992), implicating an involvement for 5HT in the disorder. 5HT receptors proposed to have an important pathological and pharmacological role in OCD include the $5HT_{1d}$ (El-Mansari et al., 1995) and $5HT_2$ (Erzegovesi et al., 1992) receptors. However, the exact nature of the PI-mobilizing $5HT_2$ receptor in OCD is unclear. Both enhancing $5HT_2$ function (Pigott et al., 1993) and reducing $5HT_2$ function (Erzegovesi et al., 1992) have been found to exacerbate or precipitate OC symptoms. Since 5HT and the $5HT_2$ receptors clearly are central to understanding the neuropathology of OCD, the exact role for this receptor in behavior and, indeed OCD, requires further clarification. Aggregate statistics for all the SSRIs reveal that 65–70% of treatment-naive OCD patients will respond at least moderately to these agents (Carpenter et al., 1996). This shortfall in clinical efficacy of the 5HT-selective agents hints of multiple neurotransmitter involvement in OCD pathology, especially of dopamine (DA). Indeed, various clinical studies (Brambilla et al., 1997; Marazziti et al., 1992), as well as putative animal models (Campbell et al., 1999; Szechtman et al., 1998) indicate the presence of a dopaminergic dysfunction in OCD.

Motor control is mutually regulated by a complex neurotransmitter network involving glutamatergic and GABAergic inputs from the motor cortex and striatum, respectively (Insel, 1992). This network is known as the cortico-striatal-thalamo-cortical (CSTC) circuit. These pathways, in turn, are modulated by D_1 and D_2 receptors innervated by the substantia nigra, and $5HT_2$ receptors from the raphae nuclei (Harvey et al., 1999; Insel, 1992). Together, these pathways are mutually responsible for controlling the output stages of the globus pallidus–thalamus and the final outflow of the thalamus to the cortex and striatum (Harvey et al., 1999; Insel, 1992). Imbalances of the CSTC circuit are associated with a number of neuropsychiatric disturbances, including OCD, Parkinson's disease, Huntington's chorea, Tourette's syndrome (Rauch and Savage, 1997), and ADHD (Faraone and Biederman, 1998). At the substantia nigra in the midbrain, as well as on presynaptic DA projections within the striatum and cortex, stimulation of $5HT_2$ receptors will result in decreased synthesis and release of DA (Kapur and Remington, 1996). This action will express itself throughout the CSTC circuit such that both gamma-amino butyric acid (GABA) and glutamate (Glu) function are modified and followed inevitably by changes in motor function. Viewing this complex interactive pathway, it becomes clear that DA and 5HT can exert profound influence over GABA and Glu activity and their resultant effects on motor behavior.

Various acute and subacute studies have demonstrated the locomotor effects of inositol (Agam et al., 1994; Cohen et al., 1997; Kofman et al., 1993, 1998). At least, three receptor mechanisms important in the physiological function of the CSTC circuit are linked to the PLC–PI pathway. These include the $5HT_2$ receptor (Levine, 1997), the metabotropic Glu (mGlu) Type 1 receptor (Ferré et al., 1999), and the D_1 receptor (Friedman et al., 1997). This suggests that dietary inositol may exert its behavioral effects, and possibly its beneficial effects on OCD, through one or more of the above PI-dependent receptors. However, its ability to exacerbate ADHD (Levine et al., 1995b) hints at a delicate balance in receptor function that is disturbed by exogenous inositol administration. Unlike OCD that is associated with orbito-frontal hyperfunction and the disinhibition of internal cues (Insel, 1992), the impulsivity, inattention, and hyperactivity that characterize ADHD appears to be associated with the hypofunction of these critical areas of the brain (Rubia et al., 1999). The disorder is also strongly associated with subcortical D_2–D_4 receptor dysfunction (Faraone and Biederman, 1998). That inositol appears to have a critical yet opposing neurobiological role in these two disorders is of interest.

The present study investigates the effects of chronic inositol exposure on striatal $5HT_2$ and D_2 receptor function, as well as the effects of prolonged inositol exposure on locomotor behavior (LB) and stereotype behavior (SB). Since the indirect dopaminergic agent, dexamphetamine (DEX), is known to precipitate various forms of SBs (Cartmell et al., 1999), yet is also therapeutically effective in ADHD (Faraone and Biederman, 1998), we also examined the effects of chronic inositol-preloading on DEX-induced behaviors. A treatment duration of 12 weeks was chosen since pharmacological treatment of OCD with SSRIs requires 10–12 weeks before clinically relevant improvement in symptoms are observed (Carpenter et al., 1996). Moreover, animal studies have found that SSRI exposure of no less than 8 weeks is required to induce desensitization of the terminal 5HT autoreceptor thereby enabling enhanced release of 5HT (El-Mansari et al., 1995).

2. Experimental procedures

2.1. Reagents and drugs

All drugs or chemicals used in this study were of the highest grade commercially available. *myo*-Inositol was purchased from Takeda Vitamin and Food USA (Wilmington, NC, USA). [^{3}H]Ketanserin and [^{3}H]spiperone were purchased from NEN Life Science Products (Boston, MA, USA). Methysergide was purchased from Novartis Pharma (Midrand, South Africa) and (+)-butaclamol from Research Biomedicals (Natick, MA, USA). DEX was purchased from Smith Kline Laboratories (Herts, UK). Tris was purchased from BDH Chemicals (Poole, England). Folin–Ciolcalteu's

phenol reagent, copper (II) sulfate 5-hydrate crystals, anhydrous sodium carbonate, and potassium sodium tartrate were purchased from E Merck (Darmstadt, Germany). Aquagel I scintillation fluid was purchased from Chemlab (Bryanston, South Africa) and sodium hydroxide pellets and hydrochloric acid 32% were purchased from SAARCHEM (Krugersdorp, South Africa).

2.2. Animals

The study protocol was approved and done in accordance with the guidelines stipulated by the Ethics Committee for Use of Experimental Animals at the Potchefstroom University for Christian Higher Education (PUCHE). All animals were maintained according to a code of ethics in research, training, diagnosis, and testing of drugs in South Africa.

Guinea pigs used in this study were bred and housed in the Animal Research Center of the PUCHE. Animals of either sex, and with a mass of between 450 and 550 g were used. The animals were housed at a constantly maintained temperature of $21 \pm 5°C$ and a humidity of $50 \pm 10\%$. Full spectrum white light (intensity of between 350 and 400 lx/m) was used in a 12-h light/dark cycle, with light being from 0500 to 1700 h.

2.3. Assessment of LB and SB

Behavioral studies were performed using a Digiscan Animal Activity Monitor (DAAM; AccuScan Instruments, Columbus, OH, USA). This automated method provides continual computerized monitoring of the animal that is more sensitive than simple observation and, above all, is without the risks of investigator bias (Sanberg et al., 1983, 1987). The cages employed in these observations are surrounded by a series of horizontal infrared light beams (16 beams spaced 2.5 cm apart), with one set of beams at ground level and a second set 10 cm above the first. This array of infrared beams enables the computerized collection of all locomotor activity by a digital analyzer that effectively determines the position of the animal 100 times/s. This high-speed analysis provides a dynamic picture of all aspects of the animal's activity throughout the observation period (AccuScan Instruments). The interruption of any beam is recorded as an activity score while interruptions of two or more consecutive beams is a movement score. In the current study, animal LB was recorded by monitoring two parameters, namely, horizontal and vertical activity. Repeated interruptions of the same beam(s) from any of the two sensor arrays are measured as stereotypic activity, while different episodes of stereotypic activity with at least a 1-s interval before the beginning of another episode is recorded as a number of stereotypic movements. These parameters record such repetitive behavior as head bobbing, grooming, paw-licking, etc. For the purpose of this study, SB was recorded by monitoring the "stereotypy count," the number of beam blocks that occur while a stereotype event is taking place, and "number of stereotypy movements," the total number of stereotype events recorded between two defined episodes (AccuScan Instruments). Previous studies have shown a significant correlation between SB as measured by the DAAM and that determined by observation (Sanberg et al., 1983, 1987). After a 2-h holding period inside a DAAM cage ($42 \times 42 \times 30$ cm), all cumulative LB and SB were recorded at intervals of 10 min over a period of 120 min. All tests were performed between 0800 and 1200 h to reduce the possible influence of the animals' diurnal cycles.

2.4. Extraction of striatum

After decapitation, the brains were rapidly removed and both striata of the guinea pig were dissected out on an ice-cooled dissection slab, and placed in 1.5-ml Eppendorf tubes. These tubes were immediately immersed in liquid nitrogen ($-120°C$) and stored at $-70°C$ until the tissue was used for the radioligand binding studies.

2.5. Radioligand binding studies

2.5.1. $5HT_2$ receptor function

The determination of striatal $5HT_2$ receptor function was performed as described by Leysen et al. (1981). Briefly, [^{3}H]ketanserin (specific activity of 66.4 Ci/mmol) and cold ligand, methysergide, were prepared in distilled, deionized water over a concentration range of 0.2–3 nM for [^{3}H]ketanserin and 0.2–3 μM for methysergide. For the determination of the total binding, 1 ml membrane suspension, 50 μl of 50 mM Tris–HCl buffer, buffered at pH 7.7 (at 25°C) with HCl, and 50 μl of [^{3}H]ketanserin were added to each tube in duplicate (total incubation volume of 1.1 ml). [^{3}H]ketanserin was added in increasing concentrations to the 10 tubes.

The incubation mixture for the determination of nonspecific binding was made up of 1 ml membrane suspension, 50 μl methysergide, and 50 μl [^{3}H]ketanserin per tube (total incubation volume of 1.1 ml). Methysergide and [^{3}H]ketanserin were added in increasing concentrations to 10 tubes in duplicate. After the radioligand had been added, the homogenate was mixed thoroughly using a vortex apparatus and incubated in a water-bath at 37°C for 15 min. After incubation, the receptor binding reaction was terminated by fast vacuum filtration through Whatman GF/B glass fiber filters (25 mm in diameter), which were saturated with buffer before filtration. The incubation tubes were washed twice with ice-cold buffer, whereafter the filters were placed in polypropylene counting tubes (Packard) containing 4.5 ml of scintillation fluid. The radioactivity on the filters (the bound radioligand) was determined using a Packard Tri-carb 4660-scintillation spectrometer. Receptor density (B_{max}) and receptor affinity (K_d) were calculated using the Combicept program (Packard, Canberra). The results were reproduced as Scatchard analyses, with receptor densities expressed as

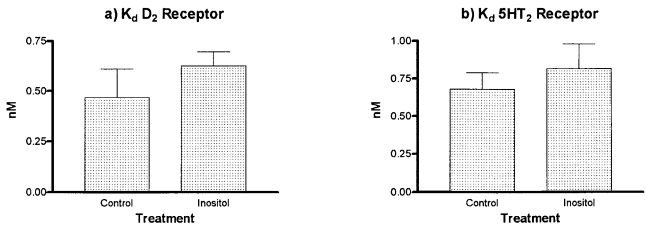

Fig. 1. (a and b) Chronic inositol-induced changes to receptor affinity (K_d; mean ± S.E.M.) for the D_2 and $5HT_2$ receptors, compared to control. Statistical significance: * $P < .05$ (Wilcoxon two-sample test).

femtomoles per milligram (fmol/mg) of protein and affinity values in nanomolar (nM).

2.5.2. D_2 receptor function

The method for the determination of D_2 receptor density in the striatum was performed as described above for the $5HT_2$ receptor, except that the D_2 selective ligands, [^{3}H]spiperone (specific activity of 16.5 Ci/mmol), and (+)-butaclamol, were used. Concentration ranges selected for the two ligands were 0.2 to 2 nM for [^{3}H]spiperone and 0.2 to 2 μM for (+)-butaclamol.

2.6. Protein determination

Samples of striatal protein were routinely assayed according to the method described by Lowry et al. (1951).

2.7. Chronic studies

Acute intraperitoneal injections of 1 g/kg inositol have been found to enhance locomotion (Kofman et al., 1993). Although much higher doses have been used (Agam et al., 1994), due the chronic nature of this study, it was decided to explore the aforementioned dosage range. Inositol (Takeda Vitamin and Food USA) was prepared by dissolving the powdered inositol in distilled, deionized water. After daily water intake was accessed, stock inositol for administration was calculated to deliver approximately 1.2 g/kg of inositol per animal per day. The animals were divided into the following groups: "control (water)," "chronic inositol," and "chronic inositol plus DEX." All the groups had free access to water and standard dietary pellets, except those groups receiving oral inositol dissolved in their water. Treatment extended over 12 weeks. At the end of the treatment period, LB and SB were determined for the "control" and "chronic inositol" groups over a period of 120 min. Finally, the latter two groups were sacrificed by carbon dioxide incapacitation followed by decapitation. Striata were immediately extracted for the determination of receptor binding data. The remaining two groups, comprising a control (water) and an inositol-treated group, then received an acute intraperitoneal injection of DEX, as described below. Each group consists of 10 animals.

2.8. Acute studies

Challenge dosages for DEX in the guinea pig were determined from dose-ranging studies previously published (Cartmell et al., 1999). DEX (3 mg/kg, ip; Cartmell et al., 1999) was used as described and found to induce significant hyperactivity and stereotypy. DEX was dissolved in normal saline. The behavioral response to acute DEX was controlled with an equal number of animals receiving intraperitoneal injection of saline. Saline (intraperitoneal) was also used as control in the acute drug challenges to the chronic inositol groups. Control and inositol-treated animals were brought into the behavioral laboratory 2 h before the experiment began, whereafter each animal received an acute intraperitoneal injection of either saline or DEX. The animals were then placed into the DAAM cages and LB and SB monitored as described earlier. Each treatment group comprised 10 guinea pigs.

2.9. Analysis of the data

All LB and SB data were first analyzed using a repeated measures analysis of variance (rmANOVA) over time and treatment over time (SAS Institute, 1988). If any significance was attained, the data were analyzed using Dunnett's post hoc test, for saline comparisons, and the Wilcoxon two-sample test for DEX vs. inositol plus DEX data (SAS Institute, 1988). Receptor binding data were analyzed using the Wilcoxon two-sample test (SAS Institute, 1988). The F test for variance (SAS Institute, 1988) was also performed on all behavioral data. In all instances, statistical significance was defined at the 95% ($P < .05$) level.

3. Results

3.1. Radioligand binding studies

3.1.1. D_2 receptor function

Chronic inositol had no effect on D_2 receptor affinity (K_d) compared to the control groups (Fig. 1a). However, a significant increase in D_2 receptor density (B_{max}) was

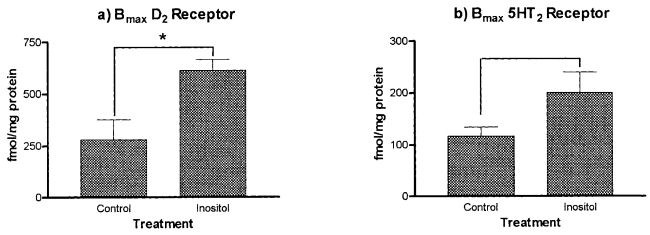

Fig. 2. (a and b) Chronic inositol-induced changes to receptor density (B_{max}; mean ± S.E.M.) for the D_2 and $5HT_2$ receptors, compared to control. Statistical significance: * $P < .05$ (Wilcoxon two-sample test).

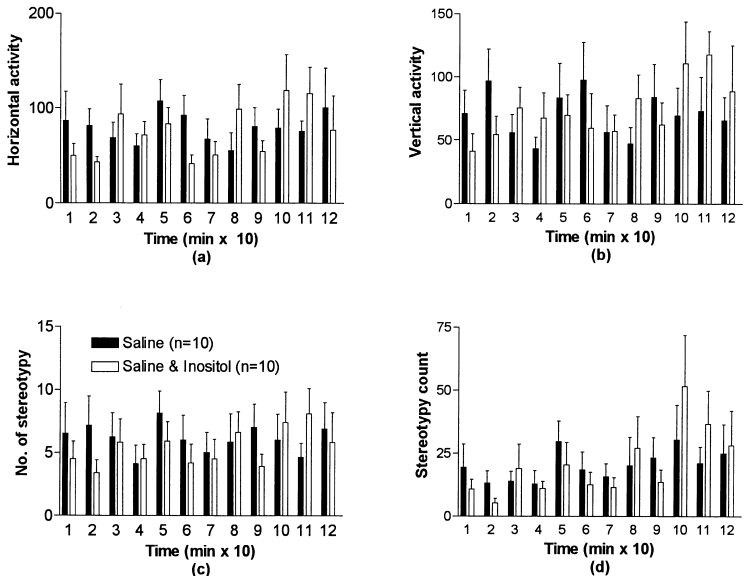

Fig. 3. (a–d) Behavioral analysis (every 10 min over 120 min) of chronic inositol compared to control on the different behavioral parameters (mean ± S.E.M.) as indicated. Statistical significance: $**P<.05$ (F test), $*P<.05$ (Dunnett's post hoc test).

observed after 3 months of inositol exposure compared to control ($P=.035$; Fig. 2a).

3.1.2. $5HT_2$ receptor function

Chronic inositol induced little change in $5HT_2$ receptor affinity (K_d) compared to the control groups (Fig. 1b). Although chronic inositol engendered a distinct rise in $5HT_2$ receptor density (B_{max}) compared to control groups, significance was not reached ($P<.1$; Fig. 2b).

3.2. Behavioral analyses

Data obtained from the rmANOVA for inositol and DEX compared to control for LB are as follows: Chronic exposure to inositol alone for 3 months did not induce any marked change in LB or SB compared to control (Fig. 3a–d). DEX induced a significant increase in vertical activity over time [$F(11,33)=4.04$; $P=.0116$] and over time and treatment [$F(11,33)=2.79$; $P=.0082$]. Effects of the drug

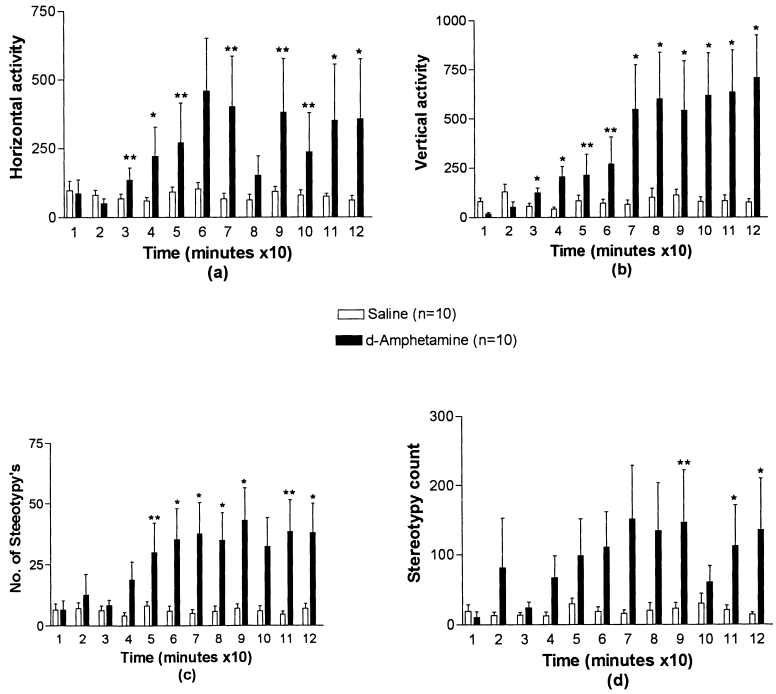

Fig. 4. (a–d) Behavioral analysis (every 10 min over 120 min) of acute DEX compared to control on the different behavioral parameters (mean ± S.E.M.) as indicated. Statistical significance: $**P<.05$ (F test), $*P<.05$ (Dunnett's post hoc test).

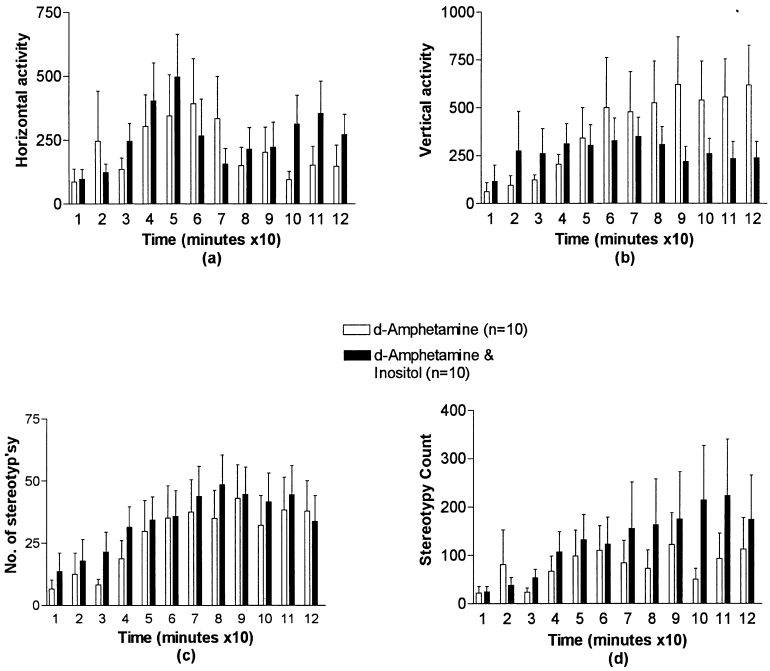

Fig. 5. (a–d) Behavioral analysis (every 10 min over 120 min) of chronic inositol plus acute DEX compared to acute DEX on the different behavioral parameters (mean ± S.E.M.) as indicated. Statistical significance: $**P<.05$ (F test), $*P<.05$ (Dunnett's post hoc test).

on horizontal activity, however, missed significance over time [$F(11,33)=2.04$; $P=.1459$] and over time and treatment [$F(11,33)=1.96$; $P=.0988$]. Acute DEX resulted in significantly raised stereotypy number over time [$F(11,33)=2.7$; $P=.019$] and over time and treatment [$F(11,33)=1.72$; $P=.0466$]. Effects on the stereotypy count over time [$F(11,33)=1.51$; $P=.2331$] and over time and treatment [$F(11,33)=1.5$; $P=.2163$] missed significance.

DEX induced LB/vertical activity over the analysis period is depicted in Fig. 4b, and in Fig. 4a for LB/horizontal activity. DEX-induced SB/number of stereotypy and SB/stereotypy count over the analysis period are depicted in Fig. 4c and d, respectively.

Chronic inositol preloading followed by acute DEX challenge resulted in some attenuation of vertical activity over time after 60 min compared to DEX alone, although significance was not reached [$F(11,154)=2.48$; $P=.0911$], and neither when examined over time and treatment [$F(11,154)=1.84$; $P=.1687$]. Similarly, inositol plus DEX was associated with little modulation of horizontal activity compared to DEX alone over time, and significance was not reached [$F(11,154)=3.12$; $P=.0700$]. The comparison over time and treatment was less so [$F(11,154)=0.94$; $P=.3914$]. The effect of inositol on DEX-induced LB/horizontal activity and LB/vertical activity over the analysis period is depicted in Fig. 5a and b. Fig. 5b indicates the attenuating effect of inositol preloading on DEX-induced vertical activity between 60 and 120 min, although significance was not reached.

Chronic inositol plus DEX vs. DEX alone was associated with significantly altered stereotypy number over time [$F(11,143)=4.23$; $P=.0018$], although little change was seen over time and treatment [$F(11,143)=0.3$ $P=.9201$]. Viewing the stereotypy count revealed no differences between these two groups over time [$F(11,154)=2.71$; $P=.1014$] or over time and treatment [$F(11,154)=0.53$; $P=.5474$]. The effect of inositol on DEX-induced SB/number of stereotypy and SB/stereotypy count over the analysis period is depicted in Fig. 5c and d.

4. Discussion

The present study has investigated the locomotor effects of chronic inositol administration and its resultant effects on the functional activity of $5HT_2$ and D_2 receptors in the guinea pig striatum, viz. receptor affinity (K_d) and density (B_{max}). Furthermore, the effects of inositol on acute DEX-induced LB and SB were also evaluated.

Chronic inositol administration to guinea pigs for 3 months induced little effect on SB and LB. This is in agreement with studies in primates (Einat et al., 1998) where 2 weeks of inositol exposure similarly did not modify behavior. In a number of studies in rodents, however, where inositol was administered for subchronic periods of either 7 or 22 days, a significant increase in LB was observed (Cohen et al., 1997; Kofman et al., 1993, 1998). Acute high-dose inositol (12 g/kg, ip) also increases LB (Agam et al., 1994). Of great interest in this study was that distinct changes especially in the DA receptor dynamics in the striatum had occurred, in spite of the lack of obvious behavioral effects. While inositol induced little

effect on ligand affinity (K_d) of both the $5HT_2$ and D_2 receptors, it engendered a significant increase in receptor density (B_{max}) for the D_2 receptor ($P<.05$). There was also some, but insignificant, evidence for increased B_{max} for the $5HT_2$ receptor ($P<.1$). Since striatal $5HT_2$ receptors are predominantly presynaptic heteroreceptors located on the A9 nuclei as well as on more distilled nigro-striatal DA projections (Harvey et al., 1999; Kapur and Remington, 1996), any change in functional activity of this receptor will have significant impact on the nigral–striatal pathway. It is now well recognized that striatal $5HT_2$ heteroreceptor stimulation will attenuate striatal DA synthesis and release (Harvey et al., 1999; Kapur and Remington, 1996). While this may lead to a compensatory upregulation of postsynaptic D_2 receptors, the present study hints of only limited $5HT_2$ receptor involvement in the observed increase in B_{max} for the D_2 receptor after chronic inositol exposure. Einat et al. (2000) have also found that chronic inositol is not associated with changes in brain monoamines after both acute and chronic administration to rats. These points suggest a mechanism that more likely involves effects on subcellular signaling than on effects on neurotransmitter release or turnover.

PI has been implicated in the regulation of various intracellular pathways, including receptor trafficking, particularly the internalization of agonist-activated G-protein-coupled receptors (Gaidarov et al., 1999), and increasing the activity of PLC (Batty and Downes, 1995). Inositol preloading may also mediate the striatal mGlu-1 receptor-mediated modulation of D_2 receptor binding (Ferré et al., 1999). While the underlying mechanisms need clarification, the changes observed in striatal D_2 receptor function, and the importance of striatal D_1–D_2 balance (Harvey et al., 1999), will result in marked changes in activity in the CSTC circuit, and in the modulation of thalamic activation of the cortex and striatum. It is thus clear that inositol presents with a clear pharmacological rationale for modulating LB. The present study, however, suggests that its inherent ability to increase LB is primarily an observation of acute and subchronic exposure as described in earlier studies (Cohen et al., 1997; Kofman et al., 1993, 1998). However, as evinced by this study, as well as studies performed in primates (Einat et al., 1998), exposure of guinea pigs to chronic inositol treatment (in this case for 3 months) may be associated with tolerance and adaptation to these acute effects. Indeed, the behavioral effects of inositol have been associated with adaptation (Cohen et al., 1997). Chronic inositol has been found to modulate or stabilize $5HT_2$ receptor sensitivity in response to pharmacological challenge (Rahman and Neuman, 1993). The present study similarly supports an ability of chronic inositol to modulate receptor sensitivity and function, although in this case upregulation of D_2 receptor function is a more prominent feature in the unchallenged animal. In order to ascertain whether this would have an impact on LB induced by a pharmacological challenge, comparative differences in DEX-induced motor behavior were also evaluated in control animals and inositol-pretreated animals.

Acute DEX administration induced an overall increase in LB and SB, but especially with regard to vertical activity and the number of stereotype movements. These behavioral effects of DEX amount to far more than simply the release of DA and certain catecholamines. In this regard, DEX-induced SB may be associated with DA (Hernandez et al., 1987), GABA (Del Arco et al., 1998), acetylcholine (Mandel et al., 1994), and Glu release (Del Arco et al., 1999). In fact, much of the behavioral effects of DEX may be mediated by the initial release of Glu (Del Arco et al., 1999), which in turn mediates the release of DA via mGlu-1 receptors (Bruton et al., 1999). Since the striatal complex is under mutual DA and Glu control (Harvey et al., 1999), any increase in glutamatergic activity, possibly via an effect of inositol on mGlu-1 receptors, may modulate dopaminergic function indirectly (Bruton et al., 1999), and present with the biochemical and behavioral consequences of DA hyperactivity (Harvey et al., 1999). In the present study, the lack of an enhanced behavioral response to DEX in the inositol-pretreated animals, given the already heightened dopaminergic state induced by such chronic inositol exposure, suggests that more complex regulatory mechanisms are at work. While this study does not strongly support $5HT_2$–D_2 receptor interactions in the striatum of the guinea pig, it does not necessarily exclude heteroreceptor interactions. Chronic inositol did induce a slight increase in $5HT_2$ receptor density, albeit not as robust as that observed with the D_2 receptor. This latter response may yet be responsible for effectively preventing inositol sensitization of DEX-induced dopaminergic behaviors, more marked in the present study especially in the vertical activity parameter. DEX has, indeed, been found to enhance the release of 5HT (Hernandez et al., 1987), which could effect such an inhibitory response on the DA function. Moreover, DEX-mediated Glu release may act on mGlu-1 receptors that will also alter the striatal D_2 receptor binding characteristics (Ferré et al., 1999).

In conclusion, the effects of chronic inositol on D_2 receptor function described in this study may hold significant interest when considering the disorders of the CSTC circuit, in particular, OCD and ADHD. We have found that chronic exposure of guinea pigs to inositol engenders a significant increase in D_2 receptor density, while having little effect on LB or SB. Since inositol has proved clinically effective in OCD (Fux et al., 1996), this observation may have relevance to OCD since earlier studies suggest a state of D_2 receptor hyporesponsiveness in the disorder (Brambilla et al., 1997). Moreover, that inositol has been found to exacerbate ADHD (Levine et al., 1995b) may also have relevance since ADHD is associated with polymorphism of the D_4 and D_2 receptor genes (Faraone and Biederman, 1998). Finally, chronic inositol exposure also exerts little, if any, effect on DEX-induced motor behavior. This may reflect complex regulation at multiple PI-mobilizing receptors within the CSTC circuit. Further study, however, is required to evaluate this

response and the effect of inositol on specific inositol-driven receptor mechanisms, e.g., D_1 $5HT_2$ and mGlu-1 receptors, using more selective receptor ligands.

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