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Repeated apomorphine administration alters dopamine D1 and D2 receptor densities in pigeon basal telencephalon

Abstract When pigeons are repeatedly administered a dose of apomorphine they show an increasing behavioral response, much as rodents do. In birds this expresses itself in an augmented pecking response. This sensitization is assumed to be largely due to a conditioning process. Here we present evidence that sensitization is accompanied by an alteration of the D_1 to D_2 dopamine receptor densities. An experimental group of pigeons was repeatedly injected with apomorphine, and a control group with saline. The basal forebrain tissue, known to be rich in dopamine receptors, was subjected to binding assays using tritiated specific D_1 and D_2 dopamine receptor antagonists. There was a trend towards an increase in D_1 and a significant decrease in D_2 receptor densities in apomorphine-treated birds compared to the saline-treated controls. We conclude that extended apomorphine treatment modifies the D_1 dopamine receptor density in the opposite manner to the D_2 dopamine receptor density.

Keywords Apomorphine · Sensitization · Dopamine receptors · Binding assay · Pigeons

Introduction

It is well known that repeated administration of a given dose of cocaine or amphetamine in rodents leads to augmentation of the locomotor activities and/or stereotyped oral responses (Anagnostaras and Robinson 1996; Lienau and Kuschinsky 1997). A similar sensitization is also observed with apomorphine, a direct and specific dopamine (DA) receptor agonist (Apo; Mattingly et al. 1997). In pigeons systemic Apo injections elicit an oral stereotypical response consisting of a prolonged bout of repetitive pecking aimed at salient but inedible features of their cage (Brunelli et al. 1975). Repeated administration of a given dose of Apo leads very reliably to a several-fold increase in the pecking response to that dose, that is to a marked behavioral sensitization to the drug (Delius 1985; Godoy 2000). Recent findings show that the sensitization to Apo in pigeons has a half-life of about two years (Keller et al. 2002).

Previously it has been shown that the sensitized response of pigeons to Apo is markedly dependent on the birds being tested in the same environmental context in which the sensitization took place (Godoy and Delius 1999; Keller and Delius 2001). This and other evidence (Wynne and Delius 1995) suggests that the pigeon's sensitization is largely due to a classical conditioning process where the drug functions as an unconditioned stimulus (US), and together with the environmental context also functions as a compound conditioned stimulus (CS) that yields a conditioned response (CR; Keller et al. 2002; Acerbo et al. 2003). Similar mechanisms are suggested to be responsible for the sensitization to psychostimulants in rodents (Anagnostaras and Robinson 1996; Lienau and Kuschinsky 1997). The conditioning hypothesis is in agreement with Wickens' (1990) proposal that a postsynaptic DA-glutamate (Glu) interaction in striatal neurons is the basis for sensory-motor learning (see also Kelley 1999). This model assumes that the DA-ergic nigrostriatal inputs function as an US and that Glu-ergic corticostriatal inputs function as a CS. Pharmacobehavioral experiments in pigeons have shown that DA receptor antagonists block the emergence of sensitization but not the expression of the corresponding CR, whereas a Glu antagonist blocks both (Acerbo et al. 2003, 2004). The co-activation of DA and Glu synapses would seem to

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selectively strengthen the efficacy of preactivated Glu synapses. The efficacy of DA synapses is assumed to remain unaffected. Nevertheless, the fact that sensitization to Apo is characterized by a change in the efficacy of this DA agonist suggests that the DA-ergic transmission might also be somehow affected. Changes in DA receptor density and affinity have been reported in connection with sensitization to cocaine in basal forebrain structures of rats (see Burechailo and Martin-Iverson 1996; Richtand et al. 1997).

The basal forebrain of birds contains structures that are thought to be equivalent to the striatum in mammals. These striatal structures are innervated by nigrotegmental DA projections and are rich in DA receptors (Durstewitz et al. 1999). Here we report that the repeated administration of Apo in pigeons leads to alterations in relative D_1 and D_2 DA receptor densities in this brain area.

Methods

Sixteen drug-naive adult pigeons (*Columba livia*) bred from local homing stock and weighing between 450 and 550 g were used. A week before the experiments began they were moved from an outside aviary to individual 40×40×45 cm stainless steel grid cages. These home cages were located in a well-ventilated and brightly-lit room (L: D 12:12 h). Animal maintenance and treatments conformed to the standards and rules laid down by the German animal welfare law. Half of the pigeons were i.m. injected daily with 1 mg/kg Apo (apomorphine hydrochloride, Teclafarm) dissolved in saline for nine days; the other half were control-injected with saline (Sal). After injection they were immediately returned to the home cage. It is well established that a sensitization to this dose of Apo, and generally to any doses between 0.2 and 2.0 mg/kg, develops strongly in these cages within some four to seven daily administrations (Wynne and Delius 1995; Acerbo et al. 2003, 2004; Acerbo and Delius 2004). Indeed, observations showed that the pecking activity of the Apo group pigeons increased from some 500 pecks/20 min on day one to about 3000 pecks/20 min on day nine, while that of the Sal group pigeons never exceeded 25 pecks/20 min, this being much as found in several other studies using Apo and Sal (Godoy 2000; Acerbo et al. 2003, 2004; Acerbo and Delius 2004). It is worth stressing that the half-life of Apo is comparatively short: in rats it has been found that the peak Apo plasma concentration occurring some 5 min after an Apo i.p. injection is reduced to half within about 20 min, and that Apo bound to striatal neural tissue has a similar survival (Martres et al. 1977; Smith et al. 1979). We have no reason to doubt that much the same is the case in pigeons. On day 11 the pigeons were killed with an overdose of pentobarbital sodium and their brains were quickly removed. The basal telencephalon (containing medial and lateral striatum, nucleus accumbens, globus pallidus and other subpallial cell groups) was dorsally and anteriorly separated along the pallial-subpallial lamina, posteriorly at the level of the preoptic nuclei and ventrally at the level of the commissura anterior (Karten and Hodos 1967; Reiner et al. 2004). The tissue was homogenized in an ice-cold 50 mM Tris-HCl (1:50 w/v) buffer (pH 7.8) and centrifuged at 48,000 g for 10 min at 4 °C. The pellets were washed with the same buffer and re-centrifuged as above. The new pellets were resuspended in 50 mM Tris-HCl buffer (pH 7.4) containing 1 mM $MgCl_2$, 2 mM $CaCl_2$, 120 mM NaCl and 5 mM KCl (incubation buffer) to yield a final protein concentration of 1 mg/ml. The saturation trials were performed using the protocol of Košťál et al. (1999). Briefly, membrane suspensions in triplicate were incubated for 30 min at 37 °C. Each tube contained 300 µl of membrane suspensions, 100 µl of [^{3}H]SCH-23390 (D_1; 70.3 Ci/mmol) in eight concentrations ranging from 0.02 to 2 nM or [^{3}H]spiperone (D2; 14.6 Ci/mmol, both DuPont NEN, USA) in seven concentrations ranging from 0.01 to 1 nM and 100 µl of incubation buffer (total binding) or either 100 µl of 1 µM SCH-23390 (D1) or 100 µM of butaclamol (D_2, both RBI, USA) (nonspecific binding). The tubes were centrifuged at 23,000 g for 6 min at 4 °C for separation of free from bound ligand. The supernatants were removed by aspiration, and the tips of the plastics tubes containing non-rinsed pellet were cut off using a heated wire and placed in scintillation vials. After the addition of scintillation liquid (SLD-41, Spolana, Czech Republic), vials were shaken for 2 h, allowed to equilibrate, and counted with a Beckman LS 6000SE scintillation counter. Protein concentrations were determined using the method of Lowry. The saturation data were analyzed using the Enzfitter program.

Results

Both [^{3}H]SCH-23390 and [^{3}H]spiperone showed saturable binding in all cases, revealing a high affinity for the target tissue. Figure 1 shows mean saturation curves of the Apo and Sal groups for D_1 and for D_2. Saturation analyses showed that the affinities of [^{3}H]SCH-23390 and of [^{3}H]spiperone did not differ significantly between the Apo and Sal group (Table 1). However, the numbers of DA receptors were partially modified. There was a statistically significant decrease in density of D_2 receptors in Apo-treated pigeons as compared to Sal-treated ones (112.9 ±13.6 fmol/mg protein against 132.8±14.8 fmol/mg protein; van der Waerden test, Z=1.99; p<0.05). There was a trend towards a higher D_1 receptor density in the Apo group than in the Sal group (169.0±3.4 fmol/mg protein against 146.3±22.4 fmol/mg protein), but this difference was not significant (van der Waerden test, Z=1.74; p=0.08). An analysis of variance proved the significant interaction between the factors receptors and treatments (two-factorial Anova, $F_{(1,12)}$=7.93; p<0.02). This interaction implies that there were diverse effects of treatment on D_1 and D_2 densities.

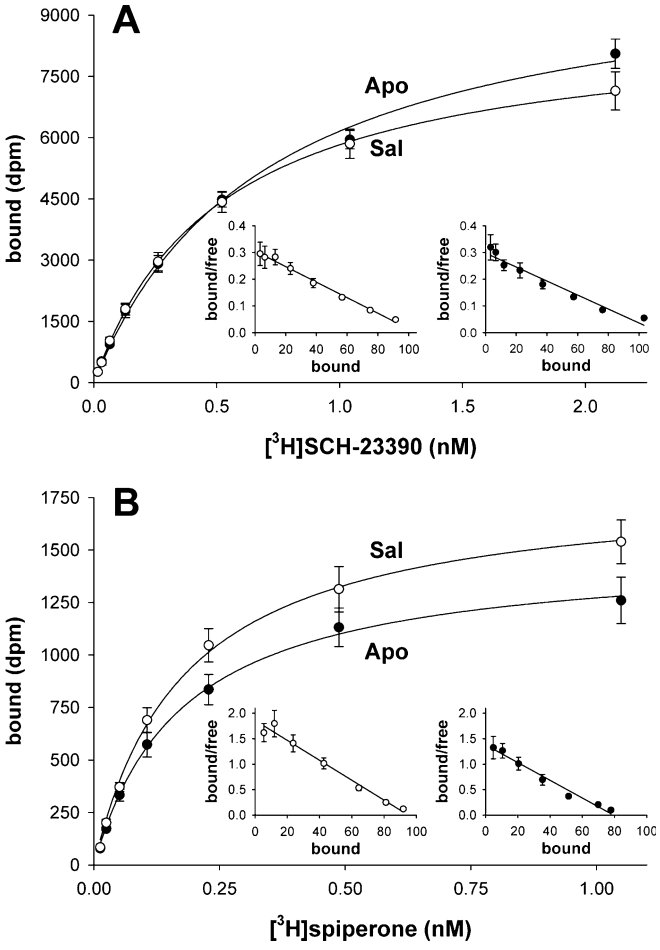

Fig. 1A B Saturation of [^{3}H]SCH 23390 (**A**) and [^{3}H]spiperone (**B**) specific binding to DA receptors in pigeon basal telencephalon homogenate, and corresponding Scatchard plots (*inserts*). Values shown are means±SEM, n 4; *filled circles* Apo group, *open circles* Sal group

Table 1 Binding parameters of [^{3}H]SCH 23390 and [^{3}H]spiperone to D_1 and D_2 DA receptors, respectively, in basal telencephalon homogenate of Apo and Sal treated pigeons

	D_1		D_2	
	K_d[a] (pM)	B_{max}[b] (fmol/mg protein)	K_d[a] (pM)	B_{max}[b] (fmol/mg protein)
Apo	462.8±78.3	169.0±3.4	62.6±7.2	112.9±13.6*
Sal	362.0±57.7	146.3±22.4	53.9±9.2	132.8±14.8

Values shown are means±SEM; n 4; [a] dissociation constant; [b] maximum binding capacity; * $p<0.05$, in comparison with Sal group

Discussion

Pharmacobehavioral studies have shown that the pecking stereotypy elicited by Apo in birds involves the activation of both D1-type and D2-type receptors (Zarrindast et al. 1992). However, in our own experiments in pigeons, when co-administered with Apo, a D2 antagonist appeared to have a stronger inhibitory effect on the behavioral sensitization that emerges upon repeated Apo injections, than a D1 antagonist did (Acerbo et al. 2003, 2004). Here, using biochemical methods, it was investigated whether exactly the same repeated Apo treatment that reliably yields a pronounced sensitization in pigeons (Godoy 2000) would lead to measurable changes in D1-type and D2-type receptor activity. We found that, indeed, that is the case: the repeated treatment with Apo had the effect of significantly decreasing the density of D2 receptors and tending to increase the density of D1 receptors. The dissociation constants of both types of receptors in the Apo pretreated pigeons were comparable to those of saline-pretreated control birds. This result suggests that the increased responsiveness that occurs upon intermittently-repeated Apo administrations in pigeons is mainly due to D2 receptor density decreases in the basal telencephalon at least. What is quite certain is that the Apo treatment in pigeons altered the relative D_1 and D_2 densities in favor of the first one.

An earlier study in rats (Chipkin et al. 1987) showed that treatments with either D1 or D2 antagonists correspondingly increased the number of D1 and D2 binding sites without affecting their affinities. However, only the animals pretreated with D2 antagonist showed a behavioral hypersensitivity upon an Apo challenge. Chipkin and colleagues concluded that the Apo induced stereotypy is mediated by both D1 and D2 receptors but that D2 receptors play a more important role in regard to sensitivity modifications. Our present finding regarding a sensitization in pigeons tends to agree with that conclusion. Rots et al. (1996) found that rats of an Apo-susceptible line had an increased number of D2 binding sites in the caudate-putamen compared with rats of an Apo-unsusceptible line. The Apo-susceptible animals conversely evinced more D1 mRNA than the unsusceptible animals but there was no difference between the lines in regard to D2 mRNA. These authors concluded that behavioral responses to Apo shown by the susceptible line were due to both a higher D2 receptor density and a higher mRNA D1 expression (Rots et al. 1996). Even though Rots and colleagues performed the DA binding and mRNA expression studies on rats without any previous Apo treatment (the Apo susceptibility of the lines was controlled in the preceding generation) our results detailed earlier appear to agree well with theirs. We too found that D2 receptor activity is essential for the Apo-induced pecking stereotypy, but we also found that the density of the D1 receptor tended to increase after Apo pretreatment. Note that in the Apo-susceptible rats there was an increased D1 mRNA expression without any accompanying D1 receptor activity increase. Other studies have also suggested that mRNA levels do not always correlate with receptor activities (Mansour et al. 1990). Possible differences in translation, in receptor processing or in post-translational events could explain such inconsistencies between the mRNA and the binding activities of a given receptor type (Mansour et al. 1990). Germeyer et al. (2002) have, however, reported that individual differences in Apo-induced stereotypes of rats probably derive from a

polymorphism of the D2 receptor gene. That polymorphism appears to concern the promotor sequence of the gene and thus regulate the gene's expression. In any case, D2 receptors appear to play a key role in Apo-induced stereotypies. However, it is also true that the modulatory role of D1 receptors cannot be denied and may indeed deserve increased attention.

The behavioral sensitization in response to repeated Apo administration in pigeons has been interpreted by us as due being to a classical conditioning process probably based on dopaminergically-aided increases in cortico-striatal glutamatergic transmission (Acerbo et al. 2003, 2004; Acerbo and Delius 2004; Godoy and Delius 1999; Keller et al. 2002; Wynne and Delius 1995). The fact that the sensitization to Apo can be blocked by a co-administration of the NMDA receptor antagonist MK-801 (Acerbo et al. 2004) suggests that the underlying neural plasticity might indeed be connected with an efficacy increase in glutamatergic synaptic transmission. A similar mechanism has also been considered in connection with the sensitization of rodents to amphetamine and cocaine, but it is probably fair to say that the evidence supporting the hypothesis is not as definite there as the evidence is in regard to Apo sensitization of pigeons (see Itzhak and Martin 2000; Mead and Stephens 1998; Szumlinski et al. 2000). Nevertheless, it seems likely that the behavioral sensitization to psychostimulant drugs involves a more elaborate mechanism than mere modifications of Glu synapses and could well implicate changes in dopaminergic transmission. This is perhaps to be expected in view of the incisive modulatory role that DA plays in basal ganglia synaptic transmission (Nicola et al. 2000).

Regardless of these considerations, we have demonstrated that sensitization to Apo in pigeons, as indexed by the pecking stereotypy that the drug elicits, is accompanied by a change in the relative D1- and D2-type receptor densities in the baso-frontal brain. It seems possible that, since Apo is a direct and specific DA agonist, and the birds evince a very specific and highly dominant response to this drug, this might be a particularly promising preparation for studying the modifications of DA synapses which arise through their intermittent but repeated activation.

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