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L-745,870 suppresses the nighttime serotonin N-acetyltransferase activity in chick retina: in vivo evidence for agonist activity at D_4-dopamine receptors

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Summary. This study examined the in vivo activity of L-745,870 at dopamine (DA) D_4 receptors, using the chick retina as a model system. In dark-adapted retinas of various vertebrates, including hen, DA acting via D_4 receptors suppresses melatonin content and activity of serotonin N-acetyltransferase (AA-NAT, a key regulatory enzyme in melatonin synthesis). Systemic administration to chicks of quinpirole (0.1 mg/kg), a high affinity agonist of D_3/D_4-DA receptors, potently decreased the nighttime AA-NAT activity of the retina. The quinpirole-evoked decline in the enzyme activity was attenuated by L-745,870 (0.1–10 nmol/eye). In addition to this action, L-745,870 given to chicks either directly into the eye (0.03–10 nmol/eye) or intraperitoneally (0.5–5 mg/kg) decreased the nighttime AA-NAT activity of the retina in a dose-dependent manner. The suppressive effect of L-745,870 on retinal AA-NAT activity was blocked by 2-chloro-11-(4-methylpiperazino)dibenz [*b*,*f*]oxepin, an antagonist of D_4-DA receptors, but was not affected by raclopride, an antagonist of D_2/D_3-DA receptors. Altogether these results indicate that in chicks L-745,870, the potent putative D_4-DA receptor antagonist, behaves in vivo as a partial D_4 agonist.

Keywords: D_4-dopamine receptor, retina, L-745,870, quinpirole, serotonin N-acetyltransferase, chick.

Introduction

Dopamine (DA) is an important neurotransmitter in the brain, being involved in the control of cognitive, emotional, motor and endocrine processes (Jackson and Westlind-Danielsson, 1994). DA is also the major catecholamine in the retina of various vertebrate species, where it functions as a neuroregulator/neurotransmitter (Djamgoz and Wagner, 1992). Physiological effects of DA are mediated by specific, membrane-bound receptors, which are

currently classified into the D1-family or D1-like (including D_1 and D_5 receptors), and the D2-family or D2-like (grouping D_2, D_3, and D_4 receptors) (Jackson and Westlind-Danielsson, 1994; Missale et al., 1997). The D2-family is of particular interest in psychiatric diseases, because antipsychotic drugs are potent antagonists of these DA receptors (Blin, 1999; Scatton and Sanger, 2000; Strange, 2001). Many neuroleptics produce, however, a number of side-effects, primarily extrapiramidal symptoms and neuroendocrine disturbances, that limit their therapeutic usefulness and reduce the patient's quality of life. Furthermore, most antipsychotic drugs are only partially effective in alleviating the negative symptoms of schizophrenia (Reynolds, 1992).

Clozapine, an atypical antipsychotic drug effective in reducing positive and negative symptoms in schizophrenic patients without eliciting extrapiramidal and neuroendocrine side-effects, displays greater affinity for D_4 over other DA receptors (Van Tool et al., 1991). This observation together with the fact that D_4 receptors are localized primarily in limbic and cortical regions of the brain (areas thought to be involved in emotional/affective behavior and cognition), and the possibility that the density of D_4 receptors may be elevated in schizophrenic patients have led to the hypothesis that blockade of central D_4 receptors plays a key role in the therapy of schizophrenia (Seeman, 1992; Matsumoto et al., 1995; Tarazi et al., 1997; Stefanis et al., 1998; Wędzony et al., 2000). This in turn led to the development of selective D_4 receptor antagonists as a new class of safer, more efficacious antipsychotic drugs (Sanner, 1998). One of such compounds was L-745,870, thought to be a selective, high affinity D_4 receptor antagonist with very good oral bioavailability and brain penetration (Kulagowski et al., 1996; Patel et al., 1997). However, in a phase II clinical trials L-745,870 was found to be ineffective in schizophrenic patients, and in some of them signs of aggravation of the illness were noticed (Bristow et al., 1997b; Kramer et al., 1997). One likely explanation of the negative outcome of these trials could be a partial agonistic activity of L-745,870 at D_4-DA receptors. In experiments performed on cloned human $D_{4.4}$- and rat D_4-DA receptors some authors reported that L-745,870 can behave as a partial agonist of these receptors (Gazi et al., 1998, 1999, 2000), while others did not detect any intrinsic activity of this compound (Kulagowski et al., 1996; Patel et al., 1997). As the ability of L-745,870 to stimulate D_4 receptors, especially in a living organism, remains a controversial issue, in the present work we have examined the in vivo activity of L-745,870 at D_4-DA receptors, using the chick retina as a model system (Zawilska and Nowak, 1997).

Material and methods

Animals

White male leghorn chicks (Hy-Line) were purchased locally on the day of hatching, and kept in temperature-controlled (29 ± 1°C during the first 5 days and 25 ± 1 °C afterwards) warmed brooders with *ad libitum* standard food and tap water. The animals were entrained to a 12-h light/12-h dark illumination cycle (LD; lights on 21:30–9:30) for a minimum of two weeks before use. The lighting cycle was produced by overhead white

cool fluorescent lamps providing light intensity at the level of animals' eyes of approximately 150 lux.

During the fourth hour of the dark phase of the LD cycle chicks received intraocular injections (i.oc.) of L-745,870 (right eye) and vehicle (left eye). The i.oc. administration was accomplished by a slow (10 sec) injection of 10 µl of appropriate solution into the vitreous body using a 30-gauge needle and Hamilton 25 µl syringe under a short lasting (up to 2 min) ether anesthesia. The animals were killed by decapitation 1 hr later. In another set of experiments, 1 hr prior to intraperitoneal (i.p.) administration of L-745,870 chicks received i.oc. injection of 2-chloro-11-(4-methylpiperazino)dibenz[*b*,*f*]oxepin (30 nmol/right eye), sulpiride (100 nmol/right eye) or raclopride (300 nmol/right eye), and vehicle (left eye). The animals were killed by decapitation 1 hr after the i.p. injection of L-745,870. In experiments on the effects of L-745,870 on the suppressive action of quinpirole on the nighttime retinal AA-NAT activity, 1 hr following the i.oc. administration of L-745,870 chicks were injected i.p. with quinpirole (0.1 mg/kg), and were killed by decapitation 1 hr later. Eyes were enucleated, hemisected at the equator, vitreous removed, neural retina dissected out and quickly frozen on dry ice. All injections and tissue dissections were performed under dim red light (4 lux). Tissues were stored at −70°C until assayed biochemically (maximally for 3 days). All experiments were carried out in strict accordance with the Polish governmental regulations concerning experiments on animals (Dz.U.97.111.724) and rules followed by the Department of Biogenic Amines.

Biochemical assays

For determination of serotonin N-acetyltransferase (AA-NAT) activity, retinas were homogenized in an ice-cold 0.05 M sodium phosphate buffer (pH 6.8) in a proportion of 1 mg wet tissue/10 µl. AA-NAT activity was measured according to the radioisotopic method previously described by us in detail (Nowak et al., 1989), using as a substrates tryptamine-HCl (1.5 mM) and acetyl coenzyme A (152 µM) containing 16 nCi [^{14}C]acetyl coenzyme A.

Chemicals

Acetyl-[1-^{14}C]-coenzyme A (sp. act. 60 mCi/mmol) was purchased from Du Pont-New England Nuclear (Boston, MA, USA). 2-Chloro-11-(4-methylpiperazino)dibenz[*b*,*f*] oxepin maleate was from Tocris Cookson Ltd. (Bristol, U.K.). L-745,870 (3-{[4-(4-chlorophenyl)piperazin-1-yl]-methyl}-1*H*pyrrolo[2.3-*b*]pyridine) and quinpirole-HCl were purchased from RBI (Natick, MA, U.S.A.). Raclopride tartate was a generous gift from Astra Research Centre AB (Sodertajle, Sweden). Sulpiride and acetyl coenzyme A disodium salt were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and tryptamine-HCl was from Serva (Heidelberg, Germany).

Quinpirole and raclopride were dissolved in 0.9% NaCl. L-745,870, 2-chloro-11-(4-methylpiperazino)dibenz[*b*,*f*]oxepin, and sulpiride were dissolved in a minimum quantity of 40% acetic acid in distilled water and diluted to final concentrations with distilled water. The final concentration of acetic acid was <0.4%. All the drugs' solutions were prepared immediately before use. Intraocular injections of 10 µl of 0.4% acetic acid in water did not affect AA-NAT activity (data not shown).

Data analysis

Data are expressed as mean ± SEM values and were analyzed for statistical significance by one-way analysis of variance followed by *post hoc* Student-Newman-Keuls test, using GraphPad Instat program (GraphPad Software, San Diego, CA, U.S.A.).

Results

In line with our earlier reports (Zawilska and Nowak, 1994), quinpirole used at a dose of 0.1 mg/kg (i.p.) potently decreased by 57–61% the nighttime AA-

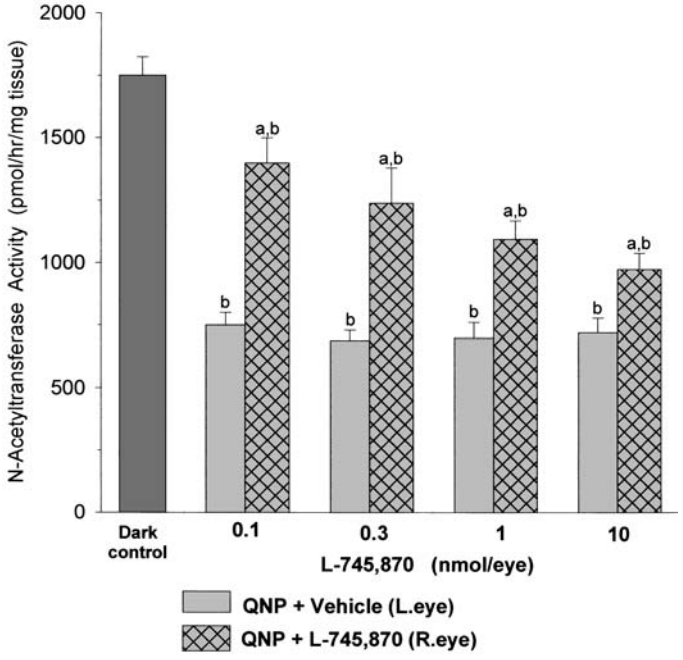

Fig. 1. Effect of L-745,870 on the quinpirole-induced suppression of the nighttime AA-NAT activity of the chick retina. At the beginning of the fourth hour of the dark phase of the LD cycle chicks received i.oc. injections of L-745,870 (0.1–10 nmol/right eye) and vehicle (left eye). Following one hour the animals were injected i.p. with quinpirole (QNP; 0.1 mg/kg), and were killed by decapitation 1 hr later. Values shown are means ± S.E.M. (N = 5–10/group). [a] $P < 0.05$ vs. Quinpirole, [b] $P < 0.05$ vs. Dark control

NAT activity of the chick retina. The quinpirole-evoked decline in the enzyme activity was attenuated by L-745,870 (0.1–10 nmol/eye) given directly into the eye. The magnitude of this L-745,870 action was dependent on the dose used, with 0.1 nmol/eye being the most effective dose and 10 nmol/eye – the last effective one (Fig. 1). The rank order of effectiveness of the tested doses of L-745,870 suggested that this compound, in addition to being a potent antagonist of retinal D_4-DA receptors, could be endowed with some intrinsic activity. Thus, in order to verify this hypothesis we examined direct effects of L-745,870 on the nighttime AA-NAT activity in the chick retina.

L-745,870 injected intraocularly (0.03–10 nmol/eye) to dark-adapted chicks decreased AA-NAT activity of the retina in a dose-dependent manner, with an ED_{50} value of 0.17 nmol/eye (Fig. 2). L-745,870 also potently decreased the nighttime AA-NAT activity of the chick retina after systemic administration, producing (after 1 hour) inhibitions of the enzyme activity in the range between 19% (0.5 mg/kg) and 48% (5 mg/kg). The suppressive effect of L-745,870 (0.5–5 mg/kg, i.p.) on the retinal AA-NAT activity was blocked by 2-chloro-11-(4-methylpiperazino)dibenz[*b*,*f*]oxepin (30 nmol/eye) and sulpiride (100 nmol/eye), but not affected by raclopride (300 nmol/eye) (Fig. 3).

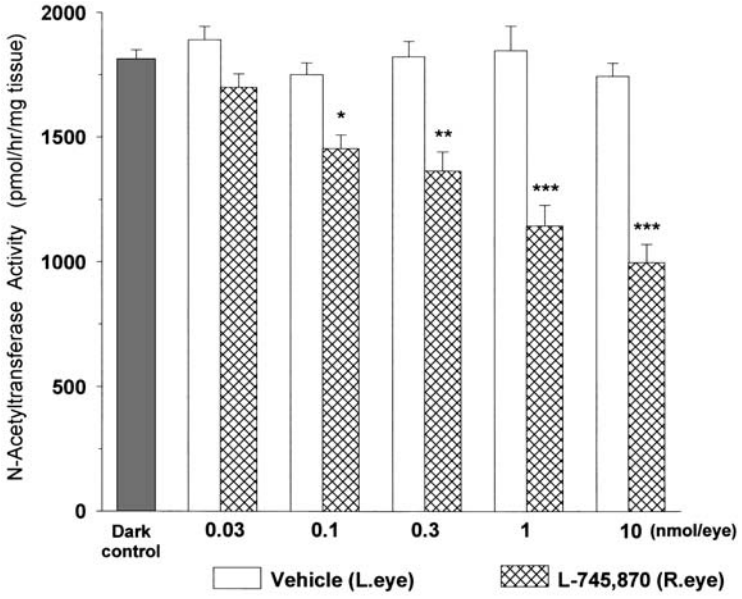

Fig. 2. Effect of L-745,870 on the nighttime AA-NAT activity of the chick retina. During the fourth hour of the dark phase of the LD cycle chicks received i.oc. injections of L-745,870 (0.03–10 nmol/right eye) and vehicle (left eye). The animals were killed by decapitation 1 hr later. Values shown are means ± S.E.M. (N = 5–6/group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Dark control

Discussion

DA, in addition to its numerous actions exerted within the brain, controls many aspects of retinal physiology (Djamgoz and Wagner, 1992). Among diverse effects of this catecholamine within the retina is the modulation of the night-driven melatonin biosynthesis, which occurs in photoreceptor cells. Activation of D_4-DA receptors rapidly suppresses the nocturnal activity of serotonin N-acetyltransferase (AA-NAT; a penultimate and key regulatory enzyme in melatonin biosynthetic pathway) and melatonin content of the retina (Zawilska, 1994; Zawilska and Nowak, 1994, 1997; Jaliffa et al., 2000; Tosini and Dirden, 2000). Interestingly, of the various brain structures analyzed thus far the retina is characterized by the highest expression of D_4 receptors (e.g., Cohen et al., 1992; Nguyen-Legros et al., 1999). With this in mind, we have previously proposed that the chick retina could be used as an easily accessible in vivo model to study potential ligands (both agonists and antagonists) of the D_4-subtype DA receptor (Zawilska and Nowak, 1997).

The present data constitute, for the first time, compelling evidence that L-745,870, a presumed highly selective antagonist of D_4 receptor (Kulagowski et al., 1996; Patel et al., 1997), acts in vivo as a D_4 agonist in the chick retina. L-745,870 mimicked the effect of quinpirole, a predominant D_3/D_4-DA receptor agonist, in suppressing the nighttime AA-NAT activity of the chick retina. 2-Chloro-11-(4-methylpiperazino)dibenz[*b*,*f*]oxepin, a potent antagonist of D_4-DA receptors (Phillips et al., 1995; Zawilska et al., 2000), and sulpiride, an

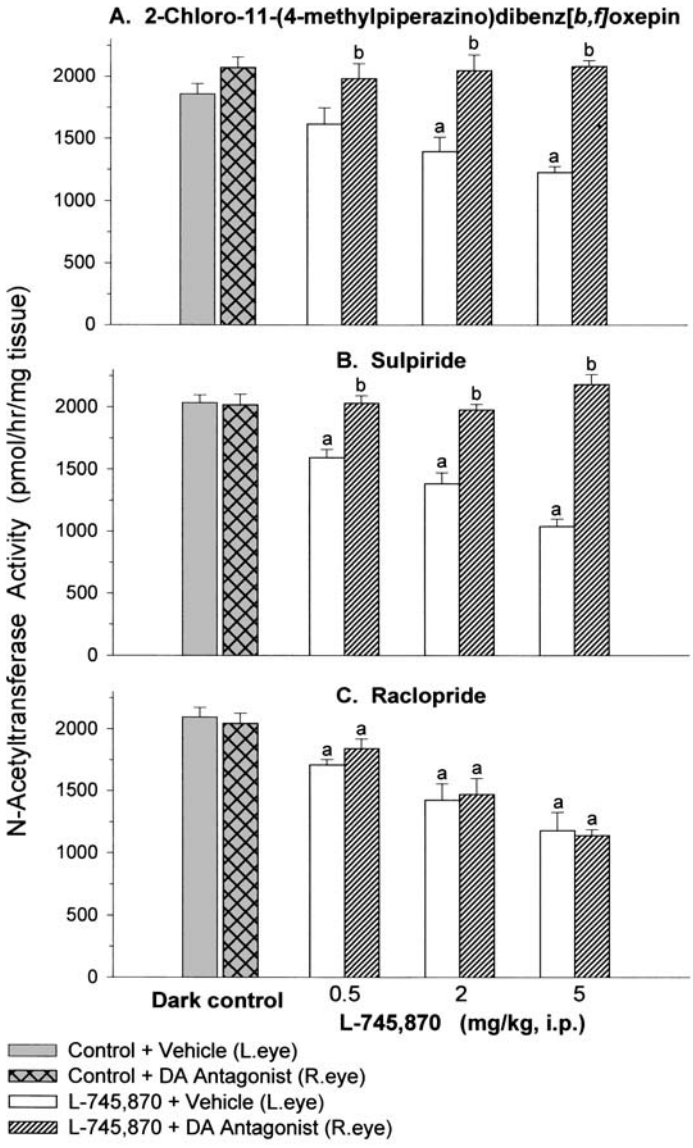

Fig. 3. Effects of antagonists of D2-family of DA receptors on the suppressive action of L-745,870 on the nighttime AA-NAT activity of the chick retina. At the beginning of the fourth hour of the dark phase of the LD cycle chicks received i.oc. injections of 2-chloro-11-(4-methylpiperazino)dibenz[*b*,*f*]oxepin (30 nmol/right eye), sulpiride (100 nmol/right eye) or raclopride (300 nmol/right eye), and vehicle (left eye). Following one hour the animals were injected i.p. with L-745,870 (0.5–5 mg/kg) or vehicle, and were killed by decapitation 1 hr later. Values shown are means ± S.E.M. (N = 5–15/group). [a] $P < 0.05$ vs. Dark control, [b] $P < 0.05$ vs L-745,870

antagonist of D2-like DA receptors (Missale et al., 1997), used at doses that effectively block D_4 receptors controlling melatonin synthesis in the chick retina (Zawilska and Nowak, 1994; Zawilska et al., 2000), abolished the suppressive action of L-745,870 on AA-NAT actiûity of the chick retina. This observation together with a lack of action of raclopride, a D_2/D_3-DA receptor

antagonist with negligible D_4 affinity (Lahti et al., 1993; Zawilska and Nowak, 1994), indicates an involvement of D_4 receptors in the studied phenomenon.

Although L-745,870 has been originally described as a potent, competitive (neutral) antagonist of recombinant human $D_{4.4}$ receptors (Kulagowski et al., 1996; Newman-Tancredi et al., 1997; Patel et al., 1997), later studies have demonstrated a meaningful intrinsic activity of this compound. Thus, in HEK293 cells expressing human $D_{4.4}$ receptors, L-745,870 inhibited forskolin-stimulated cyclic AMP accumulation in a spiperone/clozapine–sensitive and raclopride-insensitive manner. In this model L-745,870 behaved as a partial agonist, displaying 71% efficacy relative to DA (Gazi et al., 1998). Similar observations have been done on CHO cells, albeit at high density of $D_{4.4}$ receptors (Gazi et al., 1999). In behavioral studies performed on rodents L-745,870 did not exhibit an antipsychotic-like profile (Bristow et al., 1997a), and in clinical trials it failed to improve psychotic symptoms in schizophrenic patients (Bristow et al., 1997b; Kramer et al., 1997). It seems likely that this negative outcome of preclinical and clinical trials with L-745,870 could, at least partially, reflect the ability of the drug to stimulate D_4-DA receptors.

In conclusion, here we have demonstrated that L-745,870, a presumed highly selective D_4 antagonist, acts in vivo as a partial agonist of D_4 receptors in the chick retina. Our data, together with the already discussed observations done by other authors, indicate that caution should be taken when employing L-745,870 as a selective compound for studies on D_4-DA receptors.

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