ELSEVIER

European Journal of Pharmacology 295 (1996) 207–210

ejp

Short communication

The nicotinic receptor agonists (−)-nicotine and isoarecolone differ in their effects on dopamine release in the nucleus accumbens

Naheed R. Mirza [a,*], Qi Pei [b], Ian P. Stolerman [a], Tyra S.C. Zetterström [b]

[a] *Institute of Psychiatry, Section of Behavioural Pharmacology, De Crespigny Park, London SE5 8AF, UK*

[b] *Oxford University SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, UK*

Received 26 October 1995; accepted 3 November 1995

Abstract

This study compared the effect of the nicotinic receptor agonists, (−)-nicotine and isoarecolone, on the mesolimbic dopamine system of the rat using in vivo microdialysis. Previous studies showed that (−)-nicotine but not isoarecolone produced a locomotor activating effect, and that this was probably mediated by increased concentrations of dopamine in the nucleus accumbens. Nicotine (0.4 mg/kg s.c.) significantly increased extracellular concentrations of dopamine and of dihydroxyphenylacetic acid (DOPAC) by 75–80% in nucleus accumbens of rats. Isoarecolone (3.2–32 mg/kg s.c.) had no significant effect on either dopamine or DOPAC levels in this brain region and neither drug affected extracellular levels of 5-hydroxy indole acetic acid. Both nicotine and isoarecolone induced head-bobbing behaviour. Pretreatment with ketanserin reduced nicotine-induced head-bobbing suggesting a serotonergic mechanism. In conclusion, the absence of locomotor activation after administration of isoarecolone may be related to its failure to activate the mesolimbic dopamine system.

Keywords: Nicotine; Isoarecolone; Microdialysis; Dopamine; Nucleus accumbens

1. Introduction

The mesolimbic dopamine system projecting from the ventral tegmental area in the midbrain to the nucleus accumbens in the forebrain has been proposed as an important mediator of the reinforcing properties of addictive drugs and of reinforcers like food, water and intracranial electrical stimulation (Wise and Rompre, 1989). For example, amphetamine and cocaine both release dopamine in the nucleus accumbens, increase locomotor activity and are self-administered intravenously; lesions of the mesolimbic dopamine system attenuate both the locomotor activating and the reinforcing effects of these drugs. Recent studies have shown that (−)-nicotine can also release dopamine as demonstrated in vivo in freely moving rats (Imperato et al., 1986). Furthermore, nicotine preferentially induces dopamine release in the nucleus accumbens as compared with the striatum (Imperato et al., 1986) and 6-hydroxydopamine lesions of the mesolimbic dopamine system also weaken nicotine's locomotor activating and reinforcing effects (Clarke et al., 1988).

In addition to dopamine, nicotine also releases noradrenaline, acetylcholine and 5-hydroxytryptamine (5-HT) in various brain regions and different behavioural effects of nicotine have been attributed to some of these neurotransmitters, with the exception of 5-HT (Summers and Giacobini, 1995). Recently, Granon et al. (1995) reported that nicotine receptors were involved in head-bobbing behaviour in rats, but there is no information regarding the neurochemical basis for this effect.

Isoarecolone acts as a nicotinic receptor agonist at the frog neuromuscular junction and is 50- to 250-fold less potent than (−)-nicotine at inhibiting [^{3}H]nicotine binding to whole rat brain (Whiteaker et al., 1995; Stolerman, 1990). Isoarecolone has some behavioural effects that are similar to nicotine: it generalizes with the nicotine cue, depresses locomotor activity in experimentally naive rats and improves performance of rats

* Corresponding author. Tel.: 0171-919-3324; fax: 0171-703-5796; e-mail: spklnrm@ucl.ac.uk.

Elsevier Science B.V.
SSDI 0014-2999(95)00749-0

in a spatial water maze task (Stolerman, 1990; Mirza and Stolerman, 1993). However, in contrast to nicotine, isoarecolone does not increase locomotor activity in rats chronically treated with nicotine (Whiteaker et al., 1995). In view of the fact that chronic administration of nicotine increases locomotor activity by an action on the mesolimbic dopamine system, the present study compared the effects of nicotine and isoarecolone on dopamine and dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens using in vivo microdialysis.

Gross behavioural observations were made in this study to ascertain whether isoarecolone, like nicotine, induced head-bobbing behaviour in rats. Since nicotine can release 5-HT in rat cortex (Summers and Giacobini, 1995), we investigated whether this neurotransmitter played a role in nicotine-induced head-bobbing by determining whether the 5-HT_2 receptor antagonist ketanserin blocked the effect.

2. Materials and methods

2.1. Microdialysis procedure

Male Sprague-Dawley rats (220–250 g) were anaesthetized with chloral hydrate (500 mg/kg i.p.). A home made concentric dialysis probe (Sharp and Zetterström, 1992) was implanted stereotaxically into the nucleus accumbens (R +3.2 mm, L −1.2 mm, V −7.7 mm from bregma with the tooth bar set at +5.0 mm, Paxinos and Watson, 1982). The dialysis probe was secured to the skull using screws and dental cement. Following surgery rats were given 24 h to recover in their home cages with free access to food and water.

On the day of experiment rats were placed in a system for freely moving animals (CMA, Solna, Sweden) and dialysis probes were perfused with artificial cerebrospinal fluid (containing in mM: NaCl 140, KCl 3.0, $CaCl_2$ 2.4, $MgSO_4$ 1.0, Na_2HPO_4 1.2, NaH_2PO_4 0.27, glucose 7.2, pH 7.4), at 2.5 μl/min using polyethylene tubing connected to a microinfusion pump (CMA/100, Solna, Sweden). Perfusates were collected every 20 min and immediately assayed for dopamine, DOPAC and 5-hydroxy indole acetic acid (5-HIAA) content (Sharp and Zetterström, 1992) using high performance liquid chromatography coupled with electrochemical detection. Compounds were separated by reversed phase ion pair chromatography using a Microsorb C18, 5 μm column with a mobile phase comprising 0.15 M phosphate buffer, pH 3.8, containing 0.1 M EDTA, 0.5 mM sodium octane sulphonate and 12% methanol.

After an initial stable baseline was attained rats were injected with saline, nicotine (0.4 mg/kg s.c.) or isoarecolone (3.2, 10 or 32 mg/kg s.c.) followed by a delay of 10 min after which perfusates were again collected at 20 min intervals. Concentrations of dopamine, DOPAC and 5-HIAA in the dialysates were expressed as a percentage of baseline and values were given as mean ± S.E.M. Results were analyzed using two-way analysis of variance where the factors were drug (saline, nicotine or a dose of isoarecolone) and time. Tukey's honestly significant difference test was used for multiple comparisons (Unistat, UK). Rats were observed for gross behavioural changes after drug treatments.

At the end of each experiment animals were killed and brains preserved in 5% formalin. The placement of dialysis probes was verified using a freezing microtome. Only rats with probes located in the nucleus accumbens core (not shell) were included in the analysis below.

2.2. Behavioural assessments

Male Sprague-Dawley rats (220–250 g) were injected with either saline ($n = 9$) or 5 mg/kg ketanserin ($n = 5$) and then immediately placed in clear Plexiglas activity cages (Opto-Varimex II Activity Monitors; Linton Instruments, Sheffield, UK) for 20 min. Then, the rats that had been treated with saline were injected with either saline ($n = 4$) or 0.4 mg/kg of nicotine ($n = 5$); all the rats that had been treated with ketanserin were injected with 0.4 mg/kg of nicotine ($n = 5$). The animals were replaced in the cages and activity was measured for a further 20 min. During the first 15 min of this second 20-min period the number of head-bobs was counted. A head-bob was defined as a slow raising of the head with the snout pointing upward and forward, followed by a gradual lowering to the normal horizontal position. Total motor activity and the number of head-bobs were analyzed by one-way analysis of variance and Tukey's honestly significant difference test (Unistat, UK).

2.3. Drugs

Nicotine tartrate (BDH, Poole, Dorset, UK), isoarecolone HCl (donated by SmithKline Beecham, Harlow, Essex, UK) and ketanserin (Janssen, Wantage, Oxon, UK) were dissolved in 0.9% saline and administered subcutaneously in a volume of 1 ml/kg.

3. Results

3.1. Neurochemistry

The basal levels for dopamine, DOPAC and 5-HIAA in dialysates collected from nucleus accumbens were 0.063 ± 0.007, 15.8 ± 5.5 and 10.23 ± 1.33 pmol/20 min, respectively (means ± S.E.M.; $n = 23$). There were no

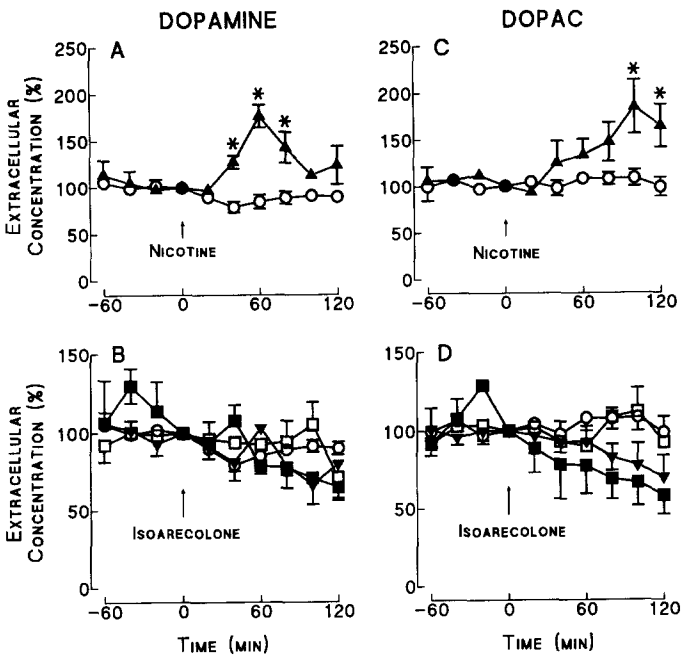

Fig. 1. Concentrations of dopamine (left column) and DOPAC (right column) in the nucleus accumbens after injection (s.c) of saline (○), nicotine 0.4 mg/kg (▲) and isoarecolone in doses of 3.2 mg/kg (■), 10 mg/kg (▼) or 32 mg/kg (□). Perfusates were collected every 20 min and once a stable baseline was attained saline, nicotine or isoarecolone was injected at the point indicated by the arrows. Perfusates were then collected at 20 min intervals for a total of 2 h. The results are expressed as a percentage of baseline. Each point is a mean ± S.E.M of 4–6 rats. $^{*}P < 0.05$ compared to saline control (Tukey's honestly significant difference test).

appreciable differences between the basal levels of these substances in the different groups.

There was a significant effect of drug ($F(4,18) = 5.71$, $P < 0.01$) and a significant drug × time interaction ($F(20,87) = 9.27$, $P < 0.001$) on extracellular dopamine levels in the nucleus accumbens. Nicotine (0.4 mg/kg) increased extracellular dopamine levels in the nucleus accumbens with a peak at 60 min (76% above baseline, Fig. 1A). Isoarecolone was without significant effect at any of the doses tested (Fig. 1B). With regard to extracellular DOPAC, there was a significant effect of drug ($F(4,18) = 4.21$, $P < 0.01$) and a drug × time interaction ($F(20,86) = 7.69$, $P < 0.001$). After injection of nicotine there was a slow rise in DOPAC levels (Fig. 1C) over the course of the 2 h period, which peaked at 100 min (84% above baseline). Isoarecolone did not increase DOPAC levels at any of the doses tested (Fig. 1D) and neither nicotine nor isoarecolone affected extracellular levels of 5-HIAA ($F(4,18) = 0.37$).

3.2. Behaviour

During the dialysis experiments, all rats injected with nicotine appeared to walk about more than controls and they displayed bouts of repetitive head-bobbing. Head-bobbing, but not increased locomotion, was also apparent in all rats injected with the larger doses of isoarecolone (10 mg/kg and 32 mg/kg). Rats injected with 32 mg/kg of isoarecolone also displayed head-weaving, front-paw treading and assumed a prostrate position. However, these behavioural effects were observed but not measured during the dialysis experiments. Since some of these behavioural effects are classical signs of the 5-HT syndrome, the effect of pretreatment with the 5-HT_2 receptor antagonist ketanserin before nicotine (0.4 mg/kg) was examined in a preliminary study.

Ketanserin had no effect on motor activity during the first 20-min period (saline, 632 ± 29 counts; ketanserin, 634 ± 46 counts). In the second 20-min period, ketanserin significantly reduced the number of head-bobs after nicotine from 40 ± 6 to 17 ± 2 ($F(2,11) = 28$, $P < 0.001$). The drug treatments also influenced locomotor activity in this period ($F(2,11) = 11.3$, $P < 0.002$); nicotine increased locomotion from 332 ± 118 counts to 792 ± 48 counts but ketanserin did not influence this response (614 ± 28 counts).

4. Discussion

The present study confirms that a systemic injection of nicotine can increase extracellular levels of dopamine and its main metabolite DOPAC in the nucleus accumbens of rats. The increase in the concentration of dopamine in the nucleus accumbens in this study was similar in magnitude to that seen in previous studies using similar doses of nicotine (Imperato et al., 1986). By contrast isoarecolone, a nicotinic receptor agonist which inhibits [^{3}H]nicotine binding (Whiteaker et al., 1995; Stolerman, 1990), did not influence either dopamine or DOPAC in the nucleus accumbens.

The lack of any effect of isoarecolone on dopamine levels in the nucleus accumbens may help to explain why this compound does not increase locomotor activity. Several lines of evidence suggest that activation of the mesolimbic dopamine system underlies the locomotor stimulation produced by nicotine. Thus, lesioning the mesolimbic dopamine system with 6-hydroxydopamine weakens nicotine's locomotor stimulant effect, and direct infusion of nicotine into the ventral tegmental area can increase locomotor activity (Clarke et al., 1988; Reavill and Stolerman, 1990). There is no reason to assume that isoarecolone does not adequately penetrate the brain as the behavioural effects it shares with nicotine are mediated centrally. Thus, at similar doses to those used in this study, isoarecolone generalizes with the nicotine discriminative stimulus which is of central origin (Kumar et al., 1987), can produce locomotor depression in naive rats (Stolerman, 1990), and similarly to nicotine has cognitive

enhancing effects in rats trained on a spatial water maze task, which is known to depend on brain structures such as the hippocampus (Mirza and Stolerman, 1993).

Although many nicotinic receptors are located on dopaminergic neurones, both in the ventral tegmental area and the nucleus accumbens (Clarke and Pert, 1985), the findings with isoarecolone suggest that it may act to some extent on a subpopulation of nicotinic receptors that are not coupled with the dopamine system. Interestingly, although the $\alpha_4\beta_2$ subunit combination is the most abundant nicotinic subtype in mammalian brain, the $\alpha_3\beta_2$ subtype may be involved in dopamine release since the nicotine receptor antagonist neuronal bungaratoxin – which has a degree of selectivity for this subtype – can block dopamine release in vitro (Grady et al., 1992). This argument is also compatible with observations that isoarecolone can produce nicotine-like discriminative stimulus effects (Stolerman, 1990), which are now thought to be independent of actions on the dopamine system (Corrigall and Coen, 1994).

The behavioural results suggested that nicotine, but not isoarecolone, increased locomotor activity. Previous studies showed that nicotine depressed locomotor activity in rats with no previous exposure to the apparatus (Stolerman, 1990). The different results in the present study may be due to procedural differences since rats in this study were habituated to activity cages for 20 min prior to nicotine injection. Differences in strain of rat or the apparatus used may also have been important. However, like nicotine, isoarecolone (10–32 mg/kg) induced bouts of head-bobbing, and at the highest dose used (32 mg/kg) also induced head-weaving, front-paw treading and prostration. Unlike isoarecolone (32 mg/kg) nicotine did not produce these additional behavioural effects at the dose used here (0.4 mg/kg), but larger doses may do so. To test for the involvement of 5-HT in nicotine-induced head-bobbing, the effect of pretreatment with the 5-HT_2 receptor antagonist ketanserin was investigated. Ketanserin significantly reduced nicotine-induced head-bobbing (0.4 mg/kg), suggesting that 5-HT_2 receptors may have been involved in the mediation of this behaviour. Although the neuroanatomical site mediating this behaviour is unknown, it is interesting that nicotine injected subcutaneously enhanced extracellular concentrations of 5-HT in rat cortex (Summers and Giacobini, 1995). Nevertheless, more extensive investigation is needed before a firm conclusion on the role of 5-HT receptors in nicotine-induced head-bobbing is reached.

In conclusion, nicotine increased the concentration of dopamine in the nucleus accumbens and induced head-bobbing behaviour whereas, over the dose range used, isoarecolone only induced head-bobbing behaviour but did not increase dopamine release, and does not stimulate locomotor activity (Whiteaker et al., 1995). As the locomotor activating effect of nicotine is mediated through the mesolimbic dopamine system, the lack of an effect of isoarecolone on dopamine in the nucleus accumbens would explain the difference between the two drugs on locomotor activity.

Acknowledgements

We thank the MRC and SmithKline Beecham for financial support.

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