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Effect of Scopolamine on the Efflux of Dopamine and Its Metabolites After Clozapine, Haloperidol or Thioridazine

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ABSTRACT

The extracellular concentrations of dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the striatum and the nucleus accumbens were measured in awake, freely-moving rats. Clozapine (20 mg/kg, i.p.) increased extracellular DA and HVA in both regions but increased DOPAC only in the striatum. Scopolamine (1 mg/kg), although it had no effect by itself in the striatum or nucleus accumbens, inhibited the ability of clozapine to increase extracellular DA, DOPAC and HVA concentrations in the striatum. The clozapine-induced increase in DA in the frontal cortex was not blocked by scopolamine. Haloperidol (1 mg/kg, i.p.) and thioridazine (10 mg/kg, i.p.) also increased extracellular DA, DOPAC and HVA in the striatum, but scopolamine pretreatment did not inhibit these increases. The results suggest that clozapine differs from haloperidol and thioridazine in that the effect of clozapine, but not that of the two neuroleptic drugs, to increase DA release in the striatum acutely depends on muscarinic receptor stimulation. These results suggest that clozapine, despite its strong muscarinic antagonist properties, does not produce full blockade of muscarinic receptors *in vivo* in the striatum. The interaction of clozapine with the cholinergic system in the striatum could be relevant to its lack of ability to produce extrapyramidal symptoms or tardive dyskinesia.

Clozapine is an atypical antipsychotic drug that does not produce catalepsy in rodents and produces significantly less acute or subacute parkinsonian symptoms and tardive dyskinesia, in man (Meltzer, 1988). Numerous theories have been proposed to explain its reduced adverse effects on the extrapyramidal system (Chiodo and Bunney, 1983; Meltzer, 1990, 1991). Initially, potent anticholinergic activity was proposed as the basis for its relative lack of interference with striatal function (Miller and Hiley, 1976; Chiodo and Bunney, 1985). Clozapine has recently been shown to have a higher affinity for the M1 human acetylcholine receptor (3.1 ± 0.7 nM) than for the M2–M5 acetylcholine receptors (Bolden *et al.*, 1991; 1992). There is considerable evidence for an anticholinergic action of clozapine *in vivo* (Racagni *et al.*, 1976; Sayers and Burki, 1976; Fjalland *et al.*, 1977; Herrling and Misbach-Lesenne, 1982; Leander, 1983). However, none of these preclinical studies clearly establish the importance of the anticholinergic effects of clozapine regarding its low EPS profile. Furthermore, clozapine produces hypersalivation in many patients (Baldessarini and Frankenburg, 1991), whereas anticholinergic drugs generally produce dry mouth. However, this effect is blocked by anticholinergic drugs (Meltzer, 1992).

Clozapine produces weak D_2 DA receptor blockade compared to the typical neuroleptic drugs (Meltzer *et al.*, 1989; Fardé *et al.*, 1989). Acute administration of the typical neuroleptic drug haloperidol increases DA release in the striatum (Imperato and DiChiara, 1985; Zetterström *et al.*, 1985; Zhang *et al.*, 1989) as well as the nucleus accumbens (Hernandez and Hoebel, 1989; Ichikawa and Meltzer, 1991) as revealed by *in vivo* microdialysis. Acute clozapine administration also increases DA release in the striatum (Imperato and Angelucci, 1989; O'Connor *et al.*, 1989) as well as in the accumbens (Invernizzi *et al.*, 1990; Moghaddam and Bunney, 1990; Ichikawa and Meltzer, 1991). The effects of clozapine on DA release are presumably due to blockade of DA autoreceptor and postsynaptic DA receptors, the latter causing stimulation of DA synthesis and release *via* a feedback loop. This increase in dopaminergic activity, coupled with weak D_2 receptor blockade by clozapine, may contribute to its low EPS profile.

Recently, Rivest and Marsden (1991), using *in vivo* differential pulse voltammetry with carbon fibre electrodes, reported that the muscarinic antagonists scopolamine (1 mg/kg, i.p.) and atropine (20 μg i.c.v.) inhibited the clozapine (30 mg/kg, i.p.)- but not the haloperidol (1 mg/kg, i.p.)-induced increases in the extracellular concentrations of the DA metabolites DOPAC, in the striatum and nucleus accumbens, of chloral hydrate-anesthetized rats. Scopolamine and atropine alone had no effect on extracellular DOPAC concentrations. The authors

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ABBREVIATIONS: DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; HPLC-ECD, high performance liquid chromatography with electrochemical detection; 5-HIAA, 5-hydroxyindoleacetic acid; CLOZ, clozapine; HAL, haloperidol; SCOP, scopolamine; VEH, vehicle.

concluded that the effect of clozapine, but not that of haloperidol, to enhance DA metabolism was dependent on intact central muscarinic receptors. They subsequently reported that scopolamine pretreatment blocked the increase in extracellular striatal DOPAC, and to a lesser extent, extracellular accumbens DOPAC, produced by i.c.v. neurotensin (Rivest and Marsden, 1992). There is other evidence for a similarity between neurotensin and clozapine. Amfonelic acid, a nonamphetamine stimulant, has been reported to block the increase in striatal DOPAC produced by clozapine and neurotensin, whereas the effects of haloperidol and perphenazine on striatal DOPAC were enhanced by pretreatment with amfonelic acid (Rivest *et al.*, 1991).

The purpose of this study was to determine, using *in vivo* microdialysis, the effect of scopolamine on the ability of clozapine, haloperidol or thioridazine to enhance extracellular DA, DOPAC and HVA concentrations of awake, freely moving rats in the striatum, nucleus accumbens and prefrontal cortex.

Materials and Methods

Animals. Male Sprague-Dawley albino rats (Zivic-Miller, PA) weighing 230 to 250 g were used throughout the study. They were housed two per cage and maintained in a controlled 12:12 hr light-dark cycle under room temperature at 22°C with free access to food and water.

Drug challenge. Scopolamine hydrochloride (1.12 mg/kg; Sigma Chemical Co., St. Louis, MO) was dissolved in physiological saline (0.9% NaCl) and administered i.p. Clozapine hydrochloride (20 mg/kg, i.p.; Sandoz), haloperidol (1 mg/kg, s.c., McNeil) and thioridazine hydrochloride (10 mg/kg, i.p., Sandoz) were dissolved in a minimal amount of 1 M tartaric acid.

Microdialysis. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p., Sigma) and mounted in a stereotaxic frame (David-Kopf). The skull was exposed. Two holes screws were inserted into the skull to secure the cemented dialysis probe tightly to the skull surface. The dialysis probes were implanted into either the striatum or the nucleus accumbens (including the dorsomedial caudate) according to the atlas of König and Klippel (1963) (lateral striatum coordinates: A +1.0, L +3.5, V −5.5; nucleus accumbens: A +3.0, L +1.5, V −7.5 mm relative to bregma); incision bar levels: −3.0 and 0 mm for the respective brain regions. The probes were cemented with quick self-curing acrylic resin (GC Unifast, Tokyo, Japan).

The microdialysis probes were of concentric flow design. Each probe was constructed by inserting a length of glass capillary tubing (150 μm, o.d.) into a piece of 26 gauge stainless steel tubing. A hollow fiber dialysis membrane (MW cutoff = 6000; 210 μm, o.d.) surrounded the exposed end of the capillary tubing and was glued to the stainless steel tubing. The exposed surface of the membrane was 2.5 mm in length for a probe in the nucleus accumbens and 3.5 mm in length for a probe in the striatum. At 18 to 20 hr after surgery, the probe was perfused with Ringer's solution (NaCl, 148 mM; Na_2HPO_4, 6.0 mM; KCl, 1.7 mM; KH_2PO_4, 1.0 mM; $CaCl_2$, 1.2 mM; pH = 7.4) at a flow rate of 2.5 μl/min by a four channel compact infusion pump (Model 975, Harvard). Monoamine levels in dialysates stabilized after 3-hr perfusion. More than four samples were collected for predrug basal values before any injection of drugs. Dialysates were collected every 30 min in microcentrifuge tubes with 25 μl of 0.8 M perchloric acid containing 20 mg/100 ml L-cysteine to prevent oxidation of monoamines in dialysates.

After obtaining stable base-line values in the dialysate, SCOP (1 mg/kg) was administered i.p. After 15 min, CLOZ (20 mg/kg) or HAL (1 mg/kg) was administered. The location of the dialysis probes was verified at the end of each experiment by dissection of the brain.

Separate groups of 5 to 7 rats were anesthetized with chloral hydrate (150 mg/kg, i.p.) and ketamine (50 mg/kg, i.p.), and a 21-gauge guide cannula was implanted dorsal to the brain surface overlying the area designated as the medial prefrontal cortex (A: +3.2; L: ±0.7 mm). Three days after implantation of the guide cannula, a dialysis probe with an exposed membrane length of 4 mm was inserted through the cannula to extend into the medial portion of the prefrontal cortex. The probe was perfused with Ringer's solutions at a flow rate of 2.0 μl/min. The dialysate collection and the drug injection paradigm were the same as described above.

HPLC-ECD analysis of striatal and accumbens dialysates. Samples were analyzed by HPLC-ECD on the day of the experiment for DA, DOPAC, HVA, and 5-HIAA after automatic injection by a refrigerated autosample processor (Kontron Model 460).

Monoamines were separated on a stainless steel, reverse phase column (Ultremex 3 C18, 3 μm, 4.6 for 75 mm, Phenomenex) at 35°C maintained by column heater and temperature controller (LC-22A, BAS). The mobile phase (pH 3.0) consisted of the prepared stock solution 0.1 M citric acid trisodium salt dihydrate (Sigma) containing EDTA-2NA (60 mg/l), Octyl sodium sulphate (30 mg/l; Kodak), and methanol (3.5–5% v/v). The sample run was about 20 min at a flow rate of 0.8 ml/min.

HPLC-ECD analysis of cortical dialysate. DA in cortical dialysates was eluted from a reverse phase column (Ultremex C18, 3 μm, 100 × 2 mm i.d.) with a mobile phase (pH 5.0) consisting of 0.1 M Na_2HPO_4, 3% CH_3CN, octyl sodium sulfate (550 mg/l) and EDTA (34 mg/l). The mobile phase was pumped through the column at a rate of 0.45 ml/min. The column temperature was maintained at 29°C. These conditions permitted the complete resolution and enhanced selectivity for DA over the catecholamine and indoleamine metabolites. Metabolites were eluted in the solvent front within 3 min and were not quantitated. DA was detected with electrochemical detection at a glassy carbon electrode maintained at +0.6 V. Sensitivity for DA was 0.3 pg/20 μl with a signal/noise ratio of 3:1.

Statistical analysis of data. The mean predrug basal levels were taken as 100% to compare the maximum mean response of DA, DOPAC and HVA after drug or vehicle. Statistical differences were determined using two-way repeated measures ANOVA and followed by post-hoc Tukey tests for multiple comparisons where appropriate. Probability within .05 was considered to indicate a significant difference.

Results

Effects of single dose of CLOZ, HAL and thioridazine on DA release and DOPAC and HVA levels in the striatum and nucleus accumbens. Basal concentrations of DA, DOPAC and HVA in the striatum were 0.095 + 0.006, 27.1 ± 1.1 and 26.3 ± 1.1 pmol/75 μl/30 min, respectively (mean ± S.E.). Basal concentrations of DA, DOPAC and HVA in the nucleus accumbens were 0.050 ± 0.004, 32.9 ± 1.36, and 22.0 ± 1.20 pmol/75 μl/30 min, respectively (mean ± S.E.).

Acute administration of CLOZ (20 mg/kg, i.p.) increased striatal and accumbens (fig. 1A–C, fig. 2A–C), extracellular DA (161 and 190%, respectively), DOPAC (163 and 115%, respectively) and HVA (181 and 200%, respectively) concentrations. There were significant differences in extracellular DA, DOPAC and HVA concentrations between VEH-CLOZ and VEH-VEH groups in the striatum ($P < .005$, $P < .001$ and $P < .001$, respectively). The effect of clozapine in the nucleus accumbens was significant only for DA and HVA ($P < .05$ and $P < .001$, respectively).

Acute administration of HAL (1 mg/kg) increased extracellular DA (212%), DOPAC (168%) and HVA (311%) concentrations in the striatum (fig. 3A–C). Differences in extracellular DA, DOPAC and HVA concentrations between VEH-HAL and VEH-VEH groups were also significant ($P < .001$, $P < .005$ and $P < .001$, respectively).

Acute administration of thioridazine also increased extracellular DA (146%), DOPAC (206%) and HVA (204%) in the

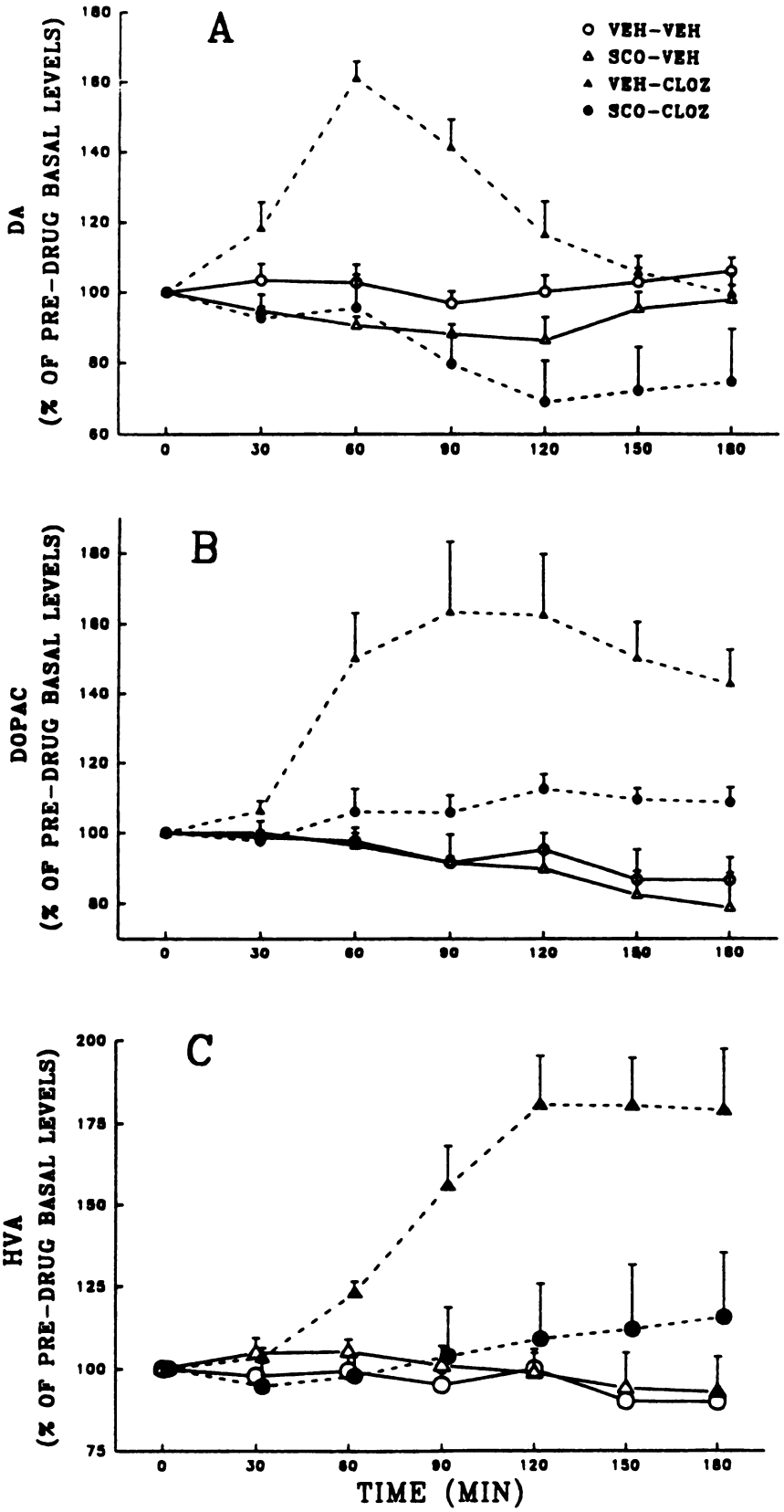

Fig. 1. The effect of scopolamine (1 mg/kg) pretreatment on: A) DA levels; B) DOPAC levels; and C) HVA levels in the striatum after clozapine (20 mg/kg) administration. Scopolamine was administered 15 min before clozapine. There was an overall significant main effect of VEH-CLOZ on DA, DOPAC and HVA. Each value is the mean ± S.E.M. of five to seven animals.

striatum (fig. 4A–C). There were significant differences in extracellular DA, DOPAC and HVA levels between VEH-thioridazine and VEH-VEH groups ($P < .005$, $P < .0001$ and $P < .001$), respectively.

Effects of single doses of scopolamine on extracellular DA, DOPAC and HVA levels. Acute administration of SCOP (1 mg/kg, i.p.) did not significantly effect striatal or accumbens (fig. 1A–C, fig. 2A–C), extracellular DA (86 and 81%, respectively), DOPAC (79 and 92%, respectively), or HVA (93 and 105%, respectively) when compared to vehicle controls.

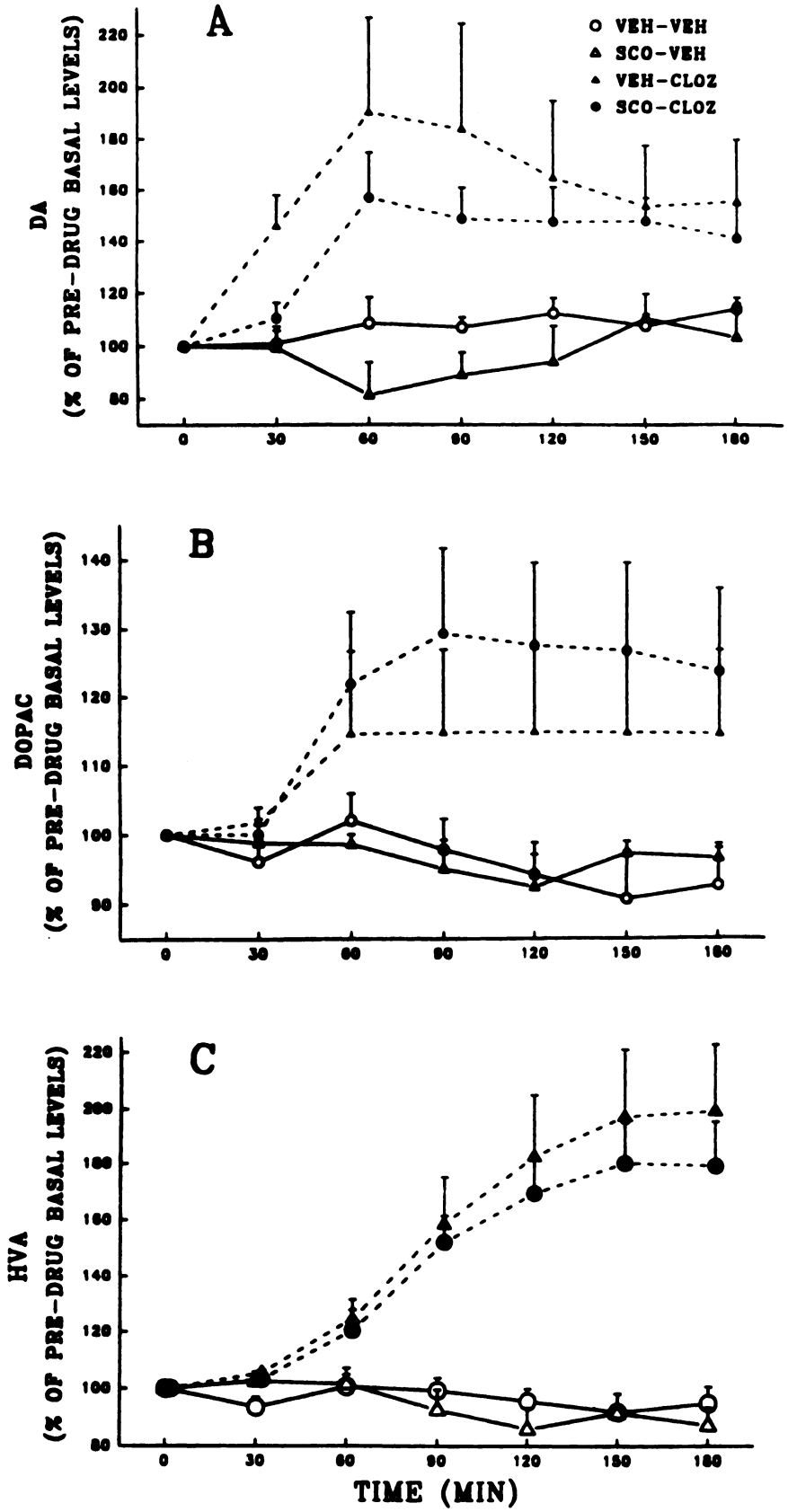

Fig. 2. The effect of scopolamine (1 mg/kg) pretreatment on: A) DA levels; B) DOPAC levels; and C) HVA levels in the nucleus accumbens after clozapine (20 mg/kg) administration. Scopolamine was administered 15 min before clozapine. There was an overall significant main effect of clozapine on DA and HVA and SCO-CLOZ on DOPAC. Each value is the mean ± S.E.M. of five to seven animals.

Effects of SCOP on CLOZ-induced DA release and DOPAC levels in the striatum and nucleus accumbens. Pretreatment with scopolamine (1 mg/kg, i.p.) 15 min before CLOZ significantly reduced CLOZ-induced DA release (96%) as well as DOPAC (113%) and HVA levels (115%) in the striatum (fig. 1A–C). There were significant differences in extracellular DA, DOPAC and HVA levels between VEH-CLOZ and SCOP-CLOZ groups ($P < .001$, $P < .005$ and $P <$

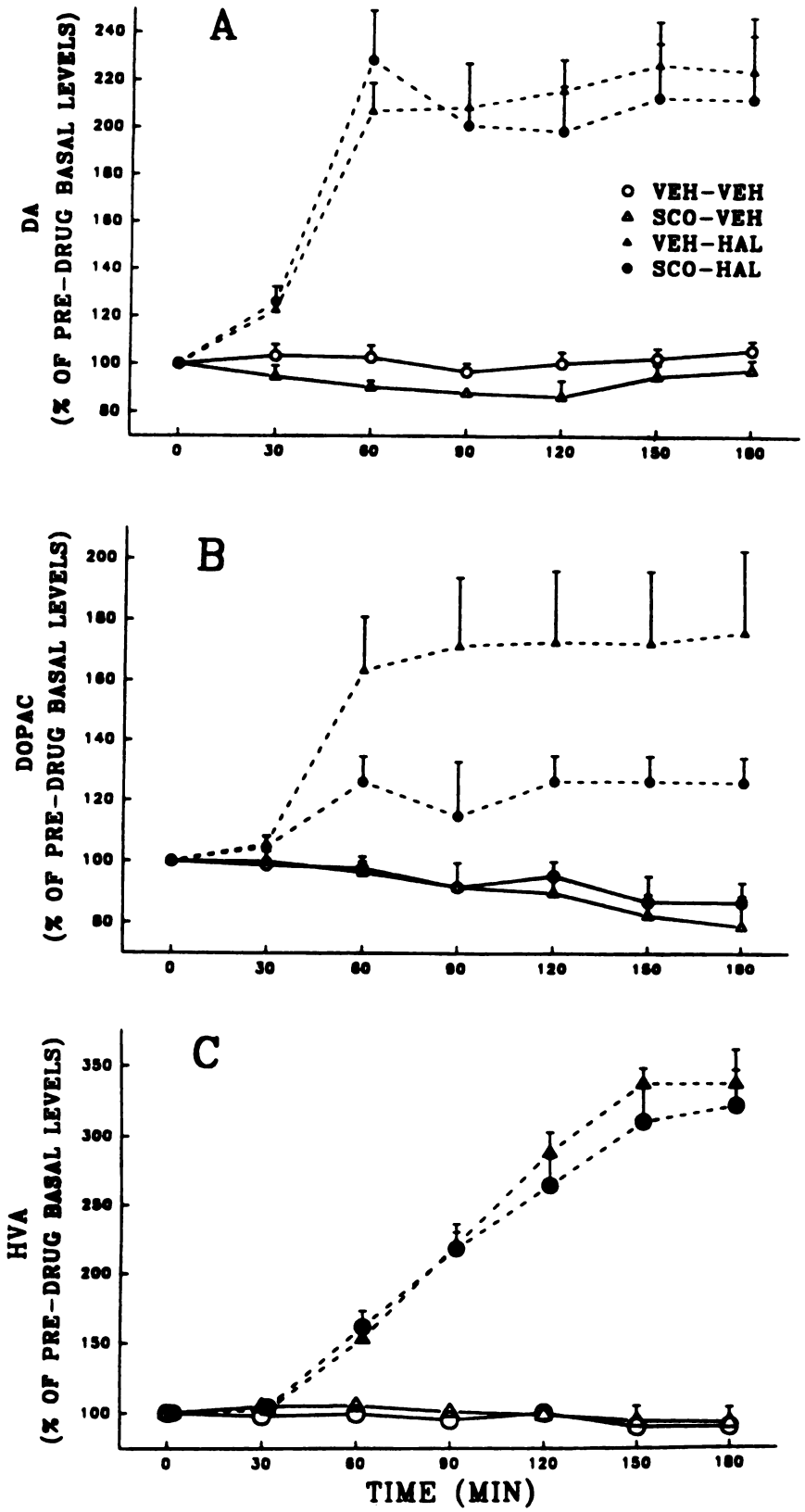

Fig. 3. The effect of scopolamine (1 mg/kg) pretreatment on: A) DA levels; B) DOPAC levels; and C) HVA levels in the striatum after haloperidol (1 mg/kg) administration. Scopolamine was administered 15 min before haloperidol. There was an overall significant main effect of VEH-HAL and SCO-HAL on DA, DOPAC and HVA. Each value is the mean ± S.E.M. of five to seven animals.

.005, respectively) in the striatum. There were no significant differences in extracellular DA, DOPAC and HVA concentrations between VEH-VEH and SCOP-CLOZ groups (data not shown).

In sharp contrast, scopolamine (1 mg/kg, i.p.) pretreatment did not significantly reduce extracellular DA, DOPAC and HVA concentrations in the nucleus accumbens of clozapine-treated rats (157, 129 and 184%, respectively) (fig. 2A–C; $P > .05$).

Effects of SCOP on HAL-induced and thioridazine DA release and DOPAC and HVA levels. SCOP (1 mg/kg, i.p.) administration 15 min before HAL had no effect on HAL-stimulated (1 mg/kg) extracellular DA or HVA concentrations (229 and 321%, respectively) in the striatum (fig. 3A–C; $P > .05$). Scopolamine (1 mg/kg, i.p.) also had no effect on the thioridazine (10 mg/kg)-stimulated concentrations of extracel-

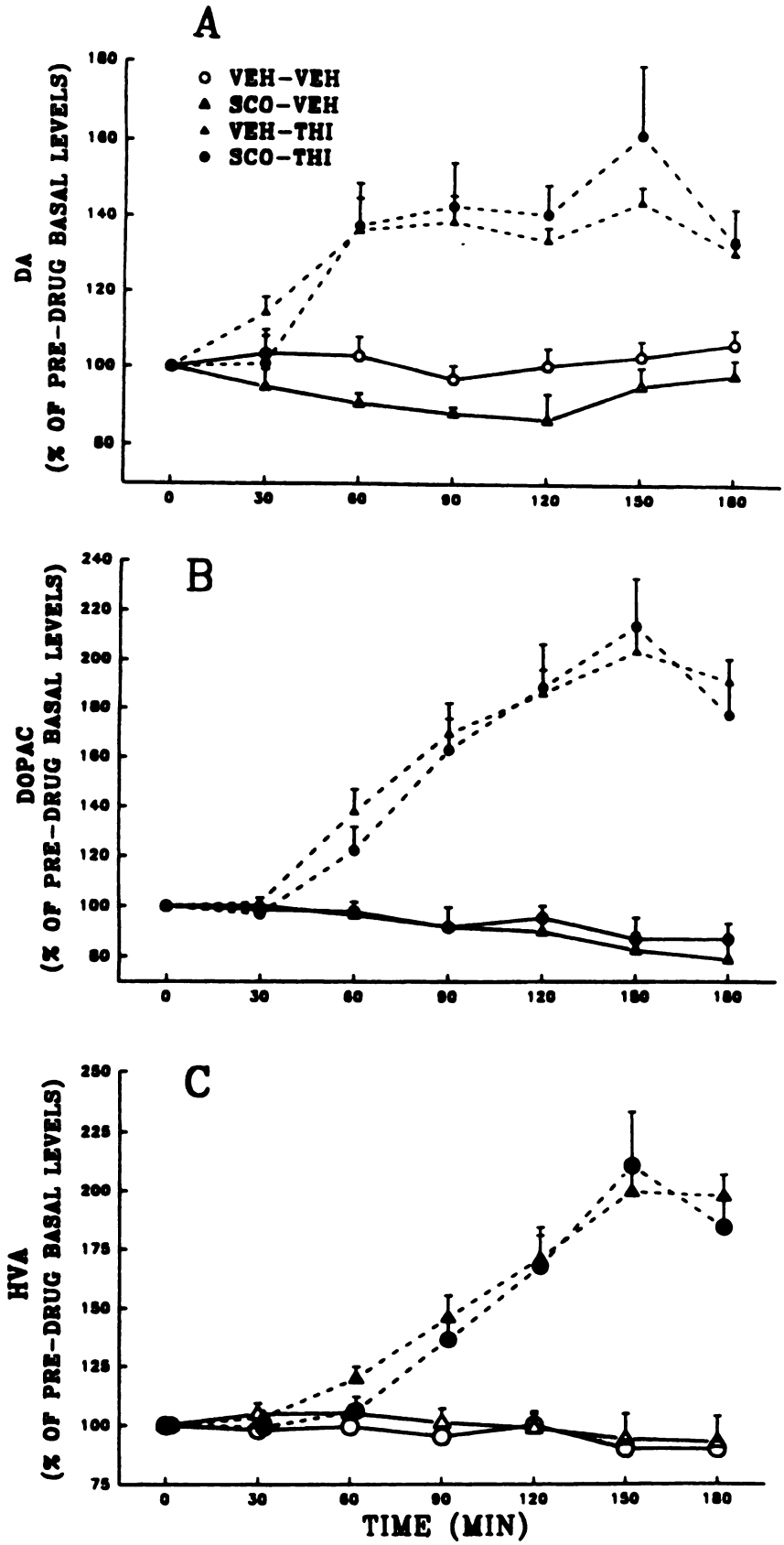

Fig. 4. The effect of scopolamine (1 mg/kg) pretreatment on: A) DA levels; B) DOPAC levels; and C) HVA levels in the striatum after thioridazine (10 mg/kg) administration. Scopolamine was administered 15 min before haloperidol. There was an overall significant main effect of VEH-THI and SCO-THI on DA, DOPAC and HVA. Each value is the mean ± S.E.M. of five to seven animals.

lular DA, DOPAC and HVA in the striatum (162, 212 and 209% at the maximum levels, respectively) (fig. 4A–C; $P > .05$).

Effect of SCOP and CLOZ on cortical DA efflux. Basal concentration of DA in dialysates collected from the medial prefrontal cortex were 0.0058 pmol/20 min. The combination of clozapine and vehicle significantly increased extracellular basal DA concentrations to 198% of base line within 1.5 hr (fig. 5). The combination of scopolamine and clozapine increased extracellular DA concentration to 227% of base line ($P < .05$). Scopolamine alone had no significant effect on the extracellular

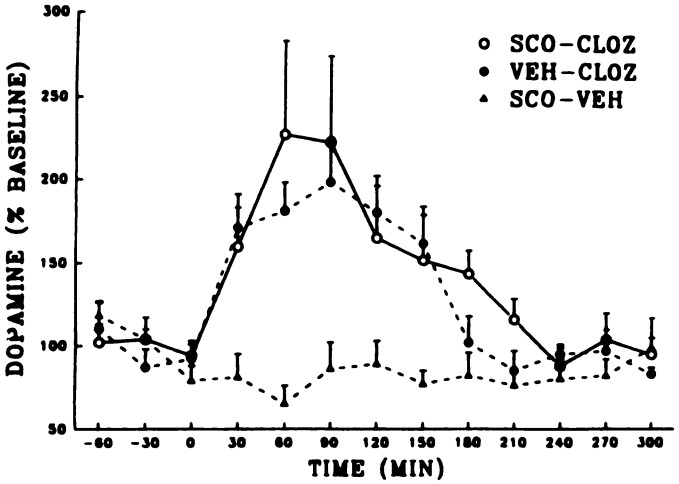

Fig. 5. Effect of scopolamine (1 mg/kg) on DA levels in the medial PFC after clozapine (20 mg/kg) administration. Scopolamine was administered 15 min before clozapine. There was an overall significant main effect of VEH-CLOZ and SCO-CLOZ on DA. Each value is the mean ± S.E.M. of six animals.

concentration of cortical DA. No significant differences were observed between the VEH-CLOZ and SCOP-CLOZ groups.

Discussion

Pretreatment with scopolamine inhibited the ability of clozapine to increase DA, DOPAC and HVA concentrations in the striatum but not the effects of clozapine to increase DA and HVA in the nucleus accumbens in awake, freely moving rats. Scopolamine also did not inhibit the clozapine-induced increase in extracellular DA in the prefrontal cortex. Scopolamine itself had no effect on basal extracellular concentrations of DA, DOPAC or HVA in the striatum or accumbens, or on extracellular DA in the frontal cortex. Scopolamine also did not affect the increase in extracellular DA, DOPAC or HVA levels produced by haloperidol or thioridazine in the striatum.

These results are in partial agreement with those of Rivest and Marsden (1991). As reported by these authors, scopolamine inhibited the ability of clozapine to increase extracellular DOPAC in the striatum in awake, freely moving rats. In addition, scopolamine was found in this study to inhibit the clozapine-induced increase in extracellular DA and HVA from the striatum. The clozapine-induced increases in DA in the striatum and the nucleus accumbens observed here were comparable to those previously reported by us (Ichikawa and Meltzer, 1991). We did not obtain data consistent with the finding of Rivest and Marsden (1991) of an effect of clozapine to increase DOPAC in the accumbens or of scopolamine to inhibit the clozapine-induced increase in DOPAC in the nucleus accumbens. Rather, scopolamine in combination with clozapine significantly increased DOPAC concentrations. Scopolamine pretreatment also did not affect the ability of clozapine to increase extracellular DA or HVA concentrations in the accumbens. Thus, these results suggest that scopolamine does not inhibit clozapine-induced DA release or metabolism in the nucleus accumbens.

It is possible that these differences may be due to the regional selectivity of the voltammetry electrode compared to the dialysis probe. Postmortem histological evaluation of the dialysis probe placements indicate that, occasionally, the membrane portion of the probe extended partially into the dorsomedial caudate in addition to the nucleus accumbens. Nevertheless, the results of the present study show a marked difference between this region and the lateral striatum in regard to the effects of scopolamine on clozapine-induced increases in extracellular DA, DOPAC and HVA.

Scopolamine alone did not affect extracellular DA, DOPAC or HVA concentrations in either striatum or accumbens. Previous studies have also found no effect of scopolamine alone to modify DA turnover in the rat striatum and nucleus accumbens, although it may decrease it in the hippocampus and frontal cortex (Memo *et al.*, 1988). Systemic atropine also had no effect on the release of DA and its metabolites in the striatum of awake, freely moving rats (Damsma *et al.*, 1988). The lack of effect of scopolamine alone on DA release in the cortex as observed here suggests that ACh does not influence DA release in the frontal cortex as well. These negative data suggest that basal DA efflux in these three regions is not regulated by a tonic cholinergic mechanism. Systemic scopolamine at doses comparable to those used here has been reported to produce a 10-fold increase in extracellular ACh concentrations in the frontal cortex and striatum of awake, freely moving rats (Tordé and Arina, 1989). Thus, increased release of ACh is not likely to contribute to the effect of scopolamine to inhibit clozapine-induced DA release.

In contrast with the lack of effect of anticholinergic drugs, cholinomimetic drugs increase DA release in the striatum (Westfall, 1974a, b; Giorguieff *et al.*, 1976, 1977; Giorguieff-Chesselet *et al.*, 1979; Gorell and Czarnecki, 1986; Xu *et al.*, 1989) and the nucleus accumbens (De Belleroche and Gardiner, 1982). The effect of ACh or cholinomimetic drugs to increase DA release may be mediated by muscarinic heteroreceptors on DA nerve terminals (Lehman and Langer, 1982; Gorell and Czarnecki, 1986). These receptors have been shown to be of the M1 subtype (Raiteri *et al.*, 1990) and, thus, should be antagonized by clozapine. Such an effect would tend to decrease the ability of clozapine to increase extracellular DA. Therefore, the effect of clozapine to increase extracellular DA concentrations must be independent of its effect to block cholinergic heteroreceptors on DA neurons. DA autoreceptors that inhibit striatal DA release (Farnebo and Hamberger, 1971; Starke *et al.*, 1978; Cubeddu and Hoffmann, 1983) are, in turn, modulated by inhibitory muscarinic and nicotinic heteroreceptors (Lehman and Langer, 1982; Raiteri *et al.*, 1982; Sakurai *et al.*, 1982), which may also be located on the same nerve terminals as the DA autoreceptors (see Gothert, 1985 for review). The ability of scopolamine pretreatment to block the increase in extracellular DA produced by clozapine in the striatum suggests that an intact presynaptic cholinergic mechanism is necessary for clozapine to effect an increase in extracellular DA in this region.

As previously mentioned, clozapine has been reported to be an effective anticholinergic agent in some (Fjalland *et al.*, 1977; Herrling and Misbach-Lesenne, 1982; Sayers and Burki, 1976; Sayers *et al.*, 1976), but not all, studies (De Jonge and Funcke, 1962). The combination of scopolamine and clozapine may achieve a greater combined inhibition of cholinergic receptors that stimulate DA release.

Clozapine itself has been reported either to have no effect or to increase ACh release. The effect of clozapine on ACh release may be dose-dependent, because 10 mg/kg i.p. did not increase ACh release *in vivo* in striatum or nucleus accumbens (Costa *et al.*, 1978), but a much higher dose (50 mg/kg, i.c.v.) increased

the release of ACh in the caudate nucleus in the gallamine-immobilized cat (Stadler *et al.*, 1974). Furthermore, clozapine (20 mg/kg, i.p.) did not increase ACh release *ex vivo* in the striatum or nucleus accumbens, nor inhibit the ability of the DA agonist TL-99 to block electrical field-stimulated ACh release from striatal and accumbens tissue slices (Compton and Johnson, 1989); however, clozapine *in vitro* at concentrations of 1 and 3 μm did reverse TL-99-induced inhibition of ACh release in the striatum and nucleus accumbens, respectively (Compton and Johnson, 1989). Acute *in vivo* administration of clozapine has been reported to produce a dose-dependent increase in the potassium stimulated release of [^{3}H]ACh from striatal slices, although treatment with clozapine for one year had the opposite effect (Kerwin *et al.*, 1984). The autoreceptor that inhibits the release of acetylcholine appears to be of the M3 subtype (De Boer *et al.*, 1990; Raiteri *et al.*, 1990). Clozapine has a strong affinity for this receptor (20 nM; Bolden *et al.*, 1991), albeit a weaker affinity than for the M1 receptor.

The possibility that increased release of DA subsequent to D_1 and/or D_2 antagonism by clozapine leads to an inhibition of ACh release must also be considered, because DA has been reported to inhibit ACh release in the striatum (Stadler *et al.*, 1973; Sethy and Van Woert, 1974; Guyenet *et al.*, 1975; Stoff *et al.*, 1982; De Boer *et al.*, 1992) and, to a lesser extent, the nucleus accumbens (Stoff *et al.*, 1987; Wedzony *et al.*, 1988; Henselmans and Stoof, 1991). Clozapine (20 mg/kg) *ex vivo* increased electrical field-stimulated DA release in the striatum but not the accumbens *in vitro*; however, it did not enhance the effect of electrical stimulation to increase DA release in the striatum *in vitro* (Compton and Johnson, 1989). It appears unlikely that an effect of clozapine on acetylcholine release is important to its ability to increase DA release in the striatum.

The inability of scopolamine itself to alter extracellular DA, DOPAC or HVA suggests that blockade of muscarinic receptors does not play a key role in the regulation of basal DA release. Therefore, the muscarinic antagonist properties of clozapine (Bolden *et al.*, 1991, 1992) are not critical to its ability to increase extracellular levels of striatal DA or to increase DA metabolism. Further, the inability of scopolamine and thioridazine to mimic the effect of scopolamine and clozapine suggests that the similar anticholinergic properties of thioridazine and clozapine are not the basis for the interaction of clozapine and scopolamine. Thioridazine and clozapine have virtually identical profiles of affinities for the human cloned ACh receptors (Bolden *et al.*, 1992). Alternatively, some other noncholinergic action of clozapine, *e.g.*, its 5-HT_2, *alpha*$_1$-adrenergic- or histamine$_1$-blocking antagonist properties, may be the basis for the ability of scopolamine to decrease clozapine-induced DA release.

There is also some evidence that clozapine is a partial cholinergic agonist at muscarinic receptors (S. Ögren, 1992, personal communication). Under basal conditions, clozapine may act as an agonist at these receptors, which would tend to stimulate DA release. However, in the presence of scopolamine these agonist properties of clozapine are blocked and, thus, interfere with the effect of clozapine to promote DA release.

A significant conclusion from the selective effect of scopolamine on the clozapine-induced increase in DA release and metabolism in the striatum, compared to the nucleus accumbens and medial prefrontal cortex, is that the cholinergic properties of clozapine may be more related to its extrapyramidal profile than to its advantages as an antipsychotic or ability to diminish negative symptoms. In this regard, it is highly relevant that scopolamine itself does not produce a similar reversal of the effect of haloperidol on extracellular DA, DOPAC or HVA concentration in either region. Thus, the lack of effect of scopolamine on the haloperidol-induced increase in DA metabolism reported by Rivest and Marsden (1991) also extends to a lack of effect on extracellular DA concentrations. Combined administration of clozapine with anticholinergic agents in man, which is a recommended means of decreasing hypersalivation due to clozapine, might be expected to alter its effects in the striatum, possibly leading acutely or chronically to more extrapyramidal symptoms because of diminution the effect of clozapine to maintain DA release in the striatum. Along these lines, the maintenance of striatal DA release has been suggested to be a critical factor in its lack of EPS (Ichikawa and Meltzer, 1990).

We confirmed the ability of clozapine to increase DA release in the cortex as previously reported by Imperato and Angelucci (1989) and Moghaddam and Bunney (1990). The lack of effect of scopolamine to reverse the clozapine-induced increase in cortical DA release indicates that there is no muscarinic receptor-sensitive effect of clozapine on DA release in this region. Thus, the mesolimbic and mesocortical basis of the effect of clozapine on extracellular DA levels are similar in this regard.

In conclusion, the present study addresses the cholinergic activity underlying the mechanism of action of clozapine. These data are suggestive of a direct and/or indirect cholinergic agonist property of clozapine. The ability of scopolamine to selectively block the clozapine-induced increases in extracellular dopamine in caudate and not nucleus accumbens or frontal cortex may have significant implications for the relative inability of clozapine to produce motor side effects. The results indicate that an intact cholinergic system is necessary for clozapine to increase dopamine release in striatum. This selective maintenance of dopamine release in striatum may contribute to its greatly diminished capacity to induce EPS. The basis for this interaction between clozapine requires further study.

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