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Effects of a psychostimulant drug Sydnocarb on rat brain dopaminergic transmission in vivo

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Abstract

Transcerebral microdialysis was used to evaluate the effect of a psychostimulant drug, sydnocarb (3-(β-phenylisopropyl)-*N*-phenylcarbamoylsydnonimine), on the extracellular levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the dorsal striatum and nucleus accumbens of freely moving rats. Sydnocarb dose dependently (4.4, 8.75 and 17.5 mg/kg, i.p.) induced a relatively modest (up to 350% of control) and long-lasting (up to 6 h) increase in dopamine extracellular level in the rat dorsal striatum. The drug at 8.75 mg/kg, i.p., produced an approximately similar increase in dopamine efflux in the dorsal striatum and in the nucleus accumbens of freely moving rats. Sydnocarb had no effect on DOPAC or HVA extracellular levels in the rat basal ganglia in vivo at any dose studied. It is important that the drug increased the efflux of dopamine in a tetrodotoxin-sensitive and Ca^{2+}-dependent manner. Measurements of behavioral parameters in non-operated rats revealed that sydnocarb markedly increased locomotor activity and induced stereotyped behavior. These data suggest that the stimulant action of sydnocarb is accompanied by a facilitation of central dopaminergic transmission involving an increase in Ca^{2+}-dependent vesicular dopamine efflux.

Keywords: Dopamine release; Microdialysis; Psychostimulant drug; Sydnocarb

1. Introduction

Sydnocarb (3-(β-phenylisopropyl)-*N*-phenylcarbamoylsydnonimine) was introduced to clinical practice in Russia as a psychostimulant drug effective for the treatment of neurasthenic symptom complex, some forms of depression, narcolepsy and attention deficit hyperactivity disorder in children (Altshuler et al., 1973; Avrutsky and Neduva, 1988). Animal studies and clinical trials have shown that sydnocarb may be effective in reducing the myorelaxant and hypnotic effects of benzodiazepines while not affecting their tranquilizing action. The efficacy of the drug in the treatment of alcohol abusers has also been reported (Rudenko and Altshuler, 1979).

Several important features of sydnocarb, which distinguish the drug from amphetamine, were found in clinical trials, namely the gradual development of a mild stimulatory effect, its long-lasting action (6–8 h), its low abuse potential, and its lack of peripheral sympathomimetic effects (Rudenko and Altshuler, 1979).

The exact mechanisms by which sydnocarb elicits psychostimulant effects are unknown; however, some evidence from animal studies indicates that the behavioral effects of sydnocarb are due, in part, to central dopaminergic mechanisms. It has previously been reported that sydnocarb, in a reserpine-sensitive manner, produces hypermotility and stereotyped behavior in rats. The drug is also effective in antagonizing neuroleptic-induced catalepsy in rats (Rudenko and Altshuler, 1979). In earlier neurochemical studies it was shown that the drug inhibits the re-uptake of dopamine and norepinephrine into rat brain synaptosomes (Erdo et al., 1981), but has no effect on monoamine oxidase activity (Rudenko and Altshuler, 1979).

In the present study we used microdialysis to test the effect of sydnocarb on dopamine release and metabolism in the dorsal striatum and nucleus accumbens of freely moving rats. The relationship of the in vivo microdialysis data to the dopamine-mediated behavioral effects of the drug was investigated by measuring, in parallel, the pattern of locomotor activity and stereotyped behavior of non-op-

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erated rats. Some of these results have been briefly reported in abstract form (Rayevsky et al., 1995; Sotnikova et al., 1995).

2. Materials and methods

2.1. Intracerebral dialysis

Male adult Wistar rats weighing 250–300 g were used. Brain dialysis was performed as previously described (Di Chiara and Imperato, 1988; Gainetdinov et al., 1994). The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. The dialysis fiber (0.32 mm outer diameter, 15000 Dalton cut off, AN 69-HF, Hospal-Dasco, Bologna, Italy) was covered with super-epoxy glue along its entire length, except for a region corresponding to the dorsal striatum and nucleus accumbens, and was held straight by a tungsten wire inside. It was implanted transversely. The coordinates used for the implantation of the microdialysis tube were as follows: dorsal striatum, AP + 2.0, V − 4.0; nucleus ac-

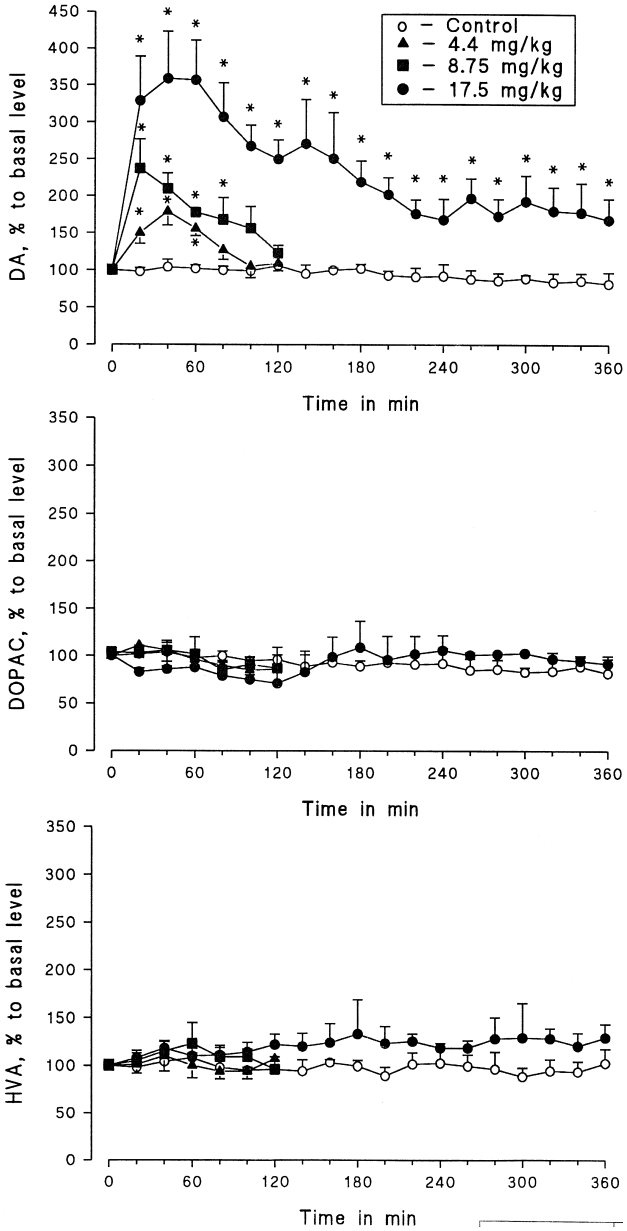

Fig. 1. Effects of sydnocarb (4.4, 8.75 and 17.5 mg/kg, i.p.) on extracellular levels of dopamine, DOPAC and HVA in the dorsal striatum of conscious rats. Data are presented as time-course changes expressed as a percentage of the mean of 3 basal samples, collected prior to injection of the drug. Means ± S.E.M. are shown ($n = 5–6$). $^{*}P < 0.05$ versus saline controls.

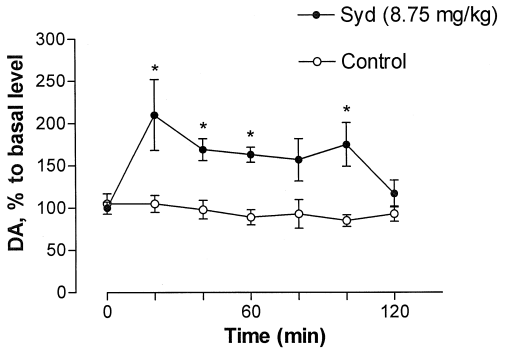

Fig. 2. Effect of sydnocarb (Syd, 8.75 mg/kg, i.p.) on dopamine efflux in the nucleus accumbens of freely moving rats. The data are expressed as percentages of the mean of 3 basal samples, collected prior to injection of the drug. Means ± S.E.M. are shown ($n = 5$). $^{*}P < 0.05$ versus saline controls.

cumbens AP + 2.5, V − 6.0, accordingly to the bregma and dura surface (Paxinos and Watson, 1982).

Twenty four hours after surgery, the dialysis probe was connected to a syringe pump and perfused at 2.7 μl/min with Ringer's solution (composition (mM) NaCl 147, $CaCl_2$ 1.5, KCl 4, pH 6.0). After a 1 h settling period the perfusate was collected every 20 min. At least four control samples were taken before drug or saline administration. Perfusate samples were assayed for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), using HPLC with electrochemical detection (BAS LC-4B, Bioanalytical System, West Lafayette, IN). Dopamine, DOPAC and HVA were separated on a reverse-phase column (Ultrasphere ODS, 5 μm, 4.6 × 150 mm), using 0.1 M citrate–phosphate mobile phase containing 1.1 mM octane sulfonic acid, 0.1 mM EDTA and 9% acetonitrile (pH 3.7), and detected by a glassy carbon working electrode set at +0.8 V.

To antagonize calcium-dependent neurotransmitter release, Ringer's solution in which Ca^{2+} was replaced by Mg^{2+} (12.5 mM) was infused through the microdialysis probe. The Mg^{2+} solutions were made by adjustment of the Na^{+} concentration (Westerink et al., 1989). To differentiate between action potential-dependent and -independent drug-induced dopamine release, tetrodotoxin (1 μM) was infused during drug treatment (Westerink et al., 1987b). Infusion of calcium antagonist or tetrodotoxin started 60 min before sydnocarb was administered.

2.2. Behavioral tests

ln a separate set of experiments non-operated rats were used to study the effect of the drug on locomotor activity and stereotyped behavior. Locomotor activity was recorded with an automated locomotor activity meter box (Rodeo-2, Russia) consisting of a square open-field arena (480 × 480 × 225 mm) equipped with three rows of photocells sensitive to infrared lights placed 40, 115 and 195 mm above the floor. The open-field was enclosed in a ventilated, sound-proof box with a Perspex top. Measurements were made in the dark between 10.00 a.m. and 4.00 p.m. The animal was habituated to the open-field box for 2 h prior to challenge with vehicle or sydnocarb. The locomotor activity of rats was determined for 5 min. Measurements were repeated each 20 min. Interruptions of horizontal movement sensors generated data that were collected automatically by an analyzer.

Immediately before and at intervals after drug administration, animals were assessed for stereotyped behavior. For this procedure, each rat was observed individually for a 5-s period at 1-min intervals over 5 consecutive min. The data are expressed as stereotypy scores, using a conventional 0–6 point stereotypy rating scale (Murray and Waddington, 1990). The testings were repeated at 20 min intervals.

2.3. Data analysis

For each group of experiments the average basal values obtained in at least three samples before drug treatment were considered as 100%. Values obtained during drug treatment were expressed as percentage of basal level. Data are presented as the means ± S.E.M. Both behavioral and microdialysis data were analyzed statistically by using the Mann–Whitney *U*-test (two-tailed). Significance at the $P < 0.05$ level and below is reported.

2.4. Drugs

In the present study sydnocarb (Chemical–Pharmaceutical Institute, Moscow) and tetrodotoxin (Sigma, USA) were used. Sydnocarb was suspended in Tween and made up to volume with saline. Tetrodotoxin was dissolved in perfusion Ringer's solution at a concentration of 1 μM.

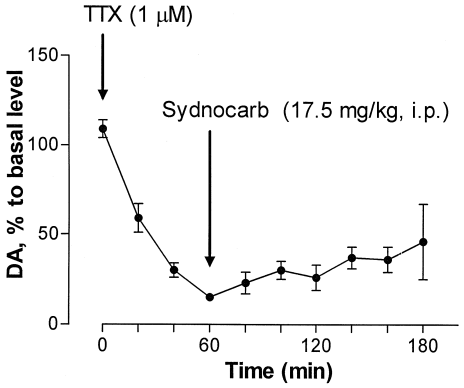

Fig. 3. Effect of sydnocarb (17.5 mg/kg, i.p.) on the dopamine level in striatal dialysates during the infusion of tetrodotoxin (TTX, 1 μM). Means ± S.E.M. are shown ($n = 5$).

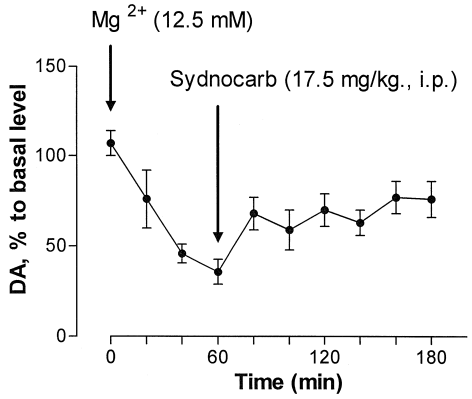

Fig. 4. Effect of sydnocarb (Syd; 17.5 mg/kg, i.p.) on the dopamine level in striatal dialysates during the perfusion of Ringer with Mg^{2+} (12.5 mM). Means ± S.E.M. are shown ($n = 5$).

The drug and saline were administered intraperitoneally in a volume of 2 ml/kg body weight.

3. Results

The basal concentrations of dopamine, DOPAC and HVA in the rat dialysates were 161.1 ± 1.7 fmol/20 min for dopamine, 33.5 ± 1.7 pmol/20 min for DOPAC and 24.5 ± 2.2 pmol/20 min for HVA from dorsal striatum (mean ± S.E.M., $n = 20$) and 115.4 ± 12.7 fmol/20 min for dopamine, 17.8 ± 0.6 pmol/20 min for DOPAC and 14.5 ± 1.5 pmol/20 min for HVA from nucleus accumbens (mean ± S.E.M., $n = 11$). Sydnocarb dose dependently (4.4, 8.75 and 17.5 mg/kg, i.p.) increased dopamine extracellular level up to 350% in the rat dorsal striatum (Fig. 1). The elevation of dialysate dopamine level following administration of the drug at 17.5 mg/kg, i.p. was found to be long-lasting (at least up to 6 h). The increase in dopamine extracellular level following sydnocarb 8.75 mg/kg, i.p. was approximately similar in the nucleus accumbens (Fig. 2) and in the dorsal striatum (Fig. 1). DOPAC and HVA extracellular levels in the rat striatum (Fig. 1) and nucleus accumbens (data not shown) were not affected by drug administration at any of the doses studied. When the sodium channel blocker tetrodotoxin (1 μM) was infused via the dialysis probe, the drug (17.5 mg/kg, i.p.) failed to increase dopamine output in the dorsal striatum of freely moving rats (Fig. 3). Similarly, replacement of Ca^{2+} by Mg^{2+} in the Ringer's solution also prevented the increase in dopamine extracellular level induced by sydnocarb (Fig. 4).

The basal level of horizontal locomotor activity of rats habituated to their environment was found to be 68 ± 15 counts per 5 min ($n = 32$). The administration of saline did not modify significantly the locomotor activity of rats. Sydnocarb markedly increased the locomotor activity of rats at all the doses studied (Fig. 5). The maximal duration

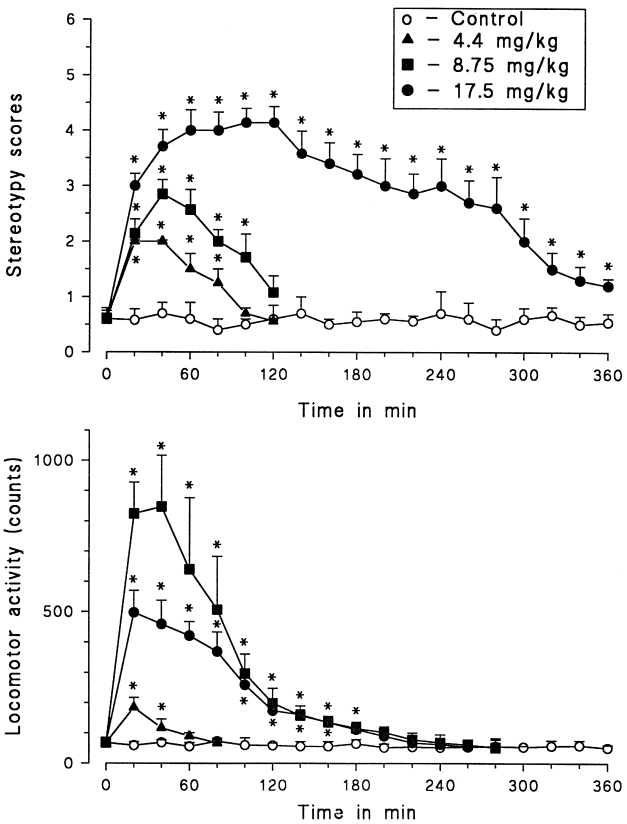

Fig. 5. Sydnocarb (4.4, 8.75 and 17.5 mg/kg, i.p.) induced stereotyped behavior and stimulation of locomotor activity of non-operated rats. Means ± S.E.M. are shown ($n = 6$). * $P < 0.05$ versus saline controls.

of hypermotility was about 3 h. The increase in locomotor activity was highest at 8.75 mg/kg, i.p.; the maximum stimulatory effect of the drug was observed 20–40 min following administration. At 17.5 mg/kg, i.p., the effect on locomotor activity was less pronounced, probably due to high level of stereotyped behavior (Fig. 5). The maximal expression and duration of stereotypy (at least up to 6 h) was observed following 17.5 mg/kg, i.p. of sydnocarb, while following administration of 4.4 and 8.75 mg/kg, i.p. the drug-induced stereotypy was relatively weak and lasted maximally 2 h.

4. Discussion

The present study demonstrates that sydnocarb in a dose- and time-dependent manner produces hyperactivity and stereotyped behavior in rats accompanied by increased dopamine efflux from dorsal striatum and nucleus accumbens. The elevation of dopamine extracellular levels in the basal ganglia of freely-moving rats following drug administration was found to be relatively mild and long-lasting. This pattern markedly differs from that produced by amphetamine (sharp massive efflux of dopamine for about 2–3 h (Sharp et al., 1987; Westerink et al., 1987b; Butcher et al., 1988; Zetterström et al., 1988; Carboni et al., 1989; Hurd and Ungerstedt, 1989; Nomikos et al., 1991; Ichikawa and Meltzer, 1992). Sydnocarb also differs from amphetamine not affecting DOPAC and HVA extracellular levels in the rat basal ganglia in vivo.

It is suggested that amphetamine induces the release of newly synthesized cytoplasmic dopamine through a carrier-mediated accelerative exchange-diffusion mechanism (Westerink et al., 1987b, 1989; Butcher et al., 1988; Zetterström et al., 1988). Therefore, amphetamine-induced decreases in extracellular DOPAC level are commonly explained as a consequence of depletion of the dopamine cytoplasmic pool that serves as a substrate for monoamine oxidase (Butcher et al., 1988; Zetterström et al., 1988; Carboni et al., 1989). In contrast, other stimulants, such as methylphenidate, nomifensine, cocaine, bupropion and GBR 12909, which increase dopamine extracellular level predominantly via inhibition of dopamine re-uptake following its physiological release from vesicular stores, have only slight effects on the extracellular DOPAC level (Westerink et al., 1987a; Zetterström et al., 1988; Carboni et al., 1989; Nomikos et al., 1989, 1990; Butcher et al., 1991).

The increase in extracellular dopamine, seen in this study, could have been produced by either increased dopamine release or inhibition of the uptake of dopamine. On the basis of earlier microdialysis studies it was suggested that the in vivo neurochemical profile of dopamine uptake inhibitors differs from that of releasers on the following criteria: gradual accumulation of extracellular dopamine (until saturation and a stable plateau level are obtained), long duration of action, and a weak dynamic effect on dopamine metabolites (Hurd and Ungerstedt, 1989). If this proposal is accepted, we would conclude that sydnocarb demonstrates the profile of a typical dopamine uptake inhibitor rather than dopamine releaser.

The mechanisms underlying the increase in the extracellular concentration of dopamine were further probed by experimental manipulation of the dopaminergic nerve terminal by using infusion of calcium antagonist or tetrodotoxin (Westerink et al., 1987b, 1989; Carboni et al., 1989). It is well known that a carrier-mediated mechanism, underlying amphetamine-induced increases of dopamine efflux, acts independently of action potentials and is insensitive to tetrodotoxin perfusion or Ca^{2+} removal (Westerink et al., 1987b, 1989; Butcher et al., 1988). In contrast, sydnocarb, like methylphenidate and nomifensine (Butcher et al., 1991), releases dopamine in a tetrodotoxin-sensitive and calcium-dependent manner. Although we did not directly examine the reserpine sensitivity of the sydnocarb-induced elevation of dopamine output, the present findings as well as earlier published data showing the reserpine-dependence of the drug-induced behavioral activation (Rudenko and Altshuler, 1979) lead us to suggest that the drug elevates extracellular dopamine levels by releasing dopamine from vesicular storage pools. The most likely mechanism underlying the increase in dopamine efflux induced by this drug therefore is inhibition of dopamine re-uptake following its physiological release via a Ca^{2+}-dependent, vesicular process. Although this explanation is consistent with earlier preliminary data (Erdo et al., 1981), the possibility that a methylphenidate-like releasing action (Ross, 1979; Zetterström et al., 1988) or other mechanisms might contribute to the observed effects of sydnocarb cannot be ruled out.

A similar potency of the drug in elevating dopamine extracellular levels in the striatum and nucleus accumbens was found. This observation is in contrast with the results of studies by Di Chiara and Imperato (1988) and Carboni et al. (1989), who reported that amphetamine and other stimulants had a more potent effect on dopamine efflux in the nucleus accumbens. It should be noted however that Sharp et al. (1987), Robinson and Camp (1990), Nomikos et al. (1989, 1991) and Ichikawa and Meltzer (1992) also did not find significant differences in the potency of amphetamine and bupropion in mesolimbic versus nigrostriatal brain regions. At present, therefore, evidence suggesting that stimulants have preferential actions on dopamine efflux in the nucleus accumbens versus striatum is equivocal.

It is concluded that the psychomotor stimulant action of sydnocarb might be due in part to facilitation of brain dopaminergic transmission involving an increase in the Ca^{2+}-dependent dopamine efflux from vesicular stores. The observed in vivo neurochemical features of sydnocarb may underlie the differences in pharmacological profile of the drug versus amphetamine-like stimulants.

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