Effects of Neuroleptics on Dopamine Release from Striatal Slices

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Summary: The effects of neuroleptics on dopamine release from rat striatal slices was investigated with a superfusion system. Haloperidol (10^{-5} M), chlorpromazine (10^{-4} M) and sulpiride (10^{-3} M) induced a significant increase in dopamine efflux from rat striatal slices. The onset of the enhancement of dopamine efflux was slower with superfusion of haloperidol than with methamphetamine. This data suggests that the mechanism of enhancement of dopamine efflux by haloperidol may be different from the mechanism of methamphetamine.

Key words: superfusion—dopamine release—striatal slices—haloperidol—chlorpromazine—sulpiride

Introduction

The neuroleptics which have antidopaminergic effects also enhanced brain dopamine (DA) turnover (Carlsson, 1963). Following administration of neuroleptics, the levels of DA metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were increased in several brain regions (Westerick, 1978) and a reduction in striatal DA fluorescence intensity was found in rats pretreated with alpha-methyl-paratyrosine (Yoshida et al. 1981). The enhancement of DA turnover by neuroleptics seemed to be caused by positive feedback due to a DA receptor blocking action, however it is unknown whether the enhancement of DA turnover is due to feedback or a direct blockade of DA autoreceptors at sites on the DA nerve endings. Attempts were made to use a superfusion apparatus to clarify the mechanism of the enhancement of DA efflux by neuroleptics. In the present study, longi-

tudinal changes of DA efflux caused by haloperidol (HPD) superfusion and drug potencies of HPD, chlorpromazine (CPZ) and sulpiride (SUL) in enhancing DA efflux were investigated.

Materials and Methods

Male Wistar rats (180-250 g) maintained in a light-, humidity- and temperature-controlled environment were used for all experiments. The rats were sacrificed by decapitation and the brain was immediately removed. Striatal slices, 0.5 mm thick were made with a Micro Slicer (Dosaka, E.M. Co.) in an ice cold Krebs solution at A 7190 to A 9650 according to the atlas of König and Klippel (1963). Striatal portions of the slices were punched out with a stainless tube (3 mm i.d.). Four slices were placed in a chamber made of teflon syringe and superfused with an O₂ saturated Krebs solution at a flow rate of 0.3

ml/min and a temperature of 37°C. The composition of the Krebs solution was (mM): NaCl, 118; KCl, 4.3; CaCl₂, 2.4; NaHCO₃, 25; NaHPO₄, 1.18; MgCl₂, 1.2 and glucose, 11. Pargyline (0.1 mM) was added to the Krebs solution to prevent degradation of DA by monoamine oxidase.

Each neuroleptic was diluted with 0.5 % tartaric acid to attain a concentration of 10⁻²M or 5 × 10⁻²M. This stock solution was diluted with Krebs solution to obtain the final concentration for each experiment. The slices were superfused for 90 min with control solution, then superfusion was continued in the presence of each concentration of drug for 20 min in the order of concentration. The concentrations of drugs were: HPD, 10⁻⁷M, 10⁻⁶M, 10⁻⁵M and 5 × 10⁻⁵M; CPZ, 10⁻⁶M, 10⁻⁵M and 10⁻⁴M; SUL, 10⁻⁵M, 10⁻⁴M and 10⁻³M. The overflow superfusate was collected in a tube in ice. The DA in the fraction was absorbed by 50 mg of alumina and eluted with 300 µl of 0.1 M phosphate buffer (pH 2.1). The eluate was injected into a high performance liquid chromatograph with an electro-chemical detector according to the method of Kissinger et al. (1977). The DA content in each fraction was expressed as percent of basal DA efflux in a fraction collected before the drug superfusion.

Results

The basal DA efflux from striatal slices superfused with Krebs solution containing 0.1 mM pargyline was 43.9 ± 8.8 pg/mg protein/min. There was no change in basal DA efflux from 90 min to 270 min after beginning the superfusion. The DOPAC efflux was less than 5 % of the DA efflux. Of the three neuroleptics used in the present study, HPD was the most potent in releasing DA from striatal slices, whereas the concentrations of CPZ and SUL necessary to induce a significant increase in DA efflux were 10⁻⁴M and 10⁻³M, respectively

Fig. 1. The effects of HPD, CPZ and SUL on endogenous DA release from rat striatal slices. Rat striatal slices were superfused for 90 min with standard Krebs solution. Superfusion was continued in the presence of each concentration of drug. The drugs were HPD (▲), CPZ (●) and SUL (○). The DA content in each fraction was expressed as percent of the basal DA efflux in the fraction obtained before the drug superfusion.

(Fig. 1). HPD (10⁻⁵M) produced a gradual increase in DA efflux during a continuous superfusion for 150 min, however, 10⁻⁵M methamphetamine (MAP) caused a rapid increase in DA efflux (Fig. 2 and 3).

Discussion

Carlsson et al. (1963) reported that neuroleptics significantly increased DA synthesis and DA turnover in the nigro-striatal DA neurons. Gamma-butyrolactone treatment (Kehr et al. 1972) or an acute lesion of the nigro-striatal pathway (Carlsson et al. 1975) failed to reduce the stimulation of DA turnover in the striatum by the neuroleptics in vivo, suggesting that

Fig. 2. Time course of DA efflux during HPD (10^{-5} M) superfusion. After superfusion with standard Krebs solution for 90 min, HPD (10^{-5} M) was added to the solution for 150 min. The superfusate was collected for 20 min at each 30 min interval.

Fig. 3. Effect of MAP on DA efflux from rat striatal slices. Striatal slices were superfused with 10^{-5} M MAP for 15 min. The superfusate was collected for 5 min at each 5 min interval.

disinhibition of the nigro-striatal pathway was not responsible for enhancement of DA efflux by the neuroleptics. The present results demonstrate on enhancement of DA efflux by the neuroleptics from striatal slices *in vitro*, which supports the earlier reports.

Anden et al. (1984) reported that the ED_{50} 's for presynaptic DA receptor blockade were 1:50:500 (HPD: CPZ: SUL) and those for postsynaptic DA receptor blockade were 1:5:37.5. For our investigation, the drug concentrations causing three fold increases in DA efflux above the basal efflux were 1:6.7:167 (HPD: CPZ: SUL).

The values obtained in the present study are close to those for the postsynaptic DA receptor blockade reported by Anden et al. (1984) suggesting that an enhancement of DA turnover by these drugs involves postsynaptic DA receptors in the striatum. Therefore, this data supports the hypothesis that blockade of the presynaptic DA autoreceptor and/or short loop disinhibition by neuroleptics is the primary mechanism to increase DA turnover. The continuous superfusion of 10^{-5} M HPD caused a gradual increase in DA efflux with a drug superfusion period of 150 min, but 10^{-5} M MAP caused a rapid increase in DA efflux (Fig. 2 and 3). The 5-fold increase in DA efflux above basal release took less than 10 min for MAP and more than 2 hours for HPD (Fig. 2 and 3). These observations suggest that the mechanisms of DA release by neuroleptics are different from the mechanism of MAP. The release of DA by MAP appears to be due to a carrier-mediated process (Raiteri et al. 1979). The delay of DA release in the presence of HPD may be due to a neuronal feedback system within the striatum, which disinhibits the release of DA from the nerve endings.

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