ELSEVIER

European Journal of Pharmacology 326 (1997) 15–21

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Duration of catalepsy correlates with increased intrastriatal sulpiride

Hiroshi P.M. Horikawa [a,b,*], Taizo Nakazato [b], Okihide Hikosaka [b]

[a] *Department of Molecular Neurobiology, Brain Research Institute, Niigata University, 1-757 Asahimachi, Niigata 951, Japan*
[b] *Department of Physiology, Juntendo University School of Medicine, Tokyo 113, Japan*

Received 9 December 1996; revised 17 February 1997; accepted 19 February 1997

Abstract

To investigate the mechanism underlying sulpiride-induced catalepsy, we simultaneously examined cataleptic behavior and the kinetics of the dopamine receptor antagonist, sulpiride of dopamine, and the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), using in vivo voltammetry. After intrastriatal administration of sulpiride to freely moving rats, the levels increased, peaked at 20 min, and remained elevated for more than 3 h. Sulpiride-induced cataleptic behavior also continued for 3 h. Levels of DOPAC peaked 180 min after the injection and did not return to baseline within the experimental period. Thus, the time-course of cataleptic behavior correlated better with elevated extracellular levels of sulpiride than with that of DOPAC. These findings suggest that sulpiride induces catalepsy via a direct action.

Keywords: Voltammetry, in vivo; Dopamine; (Rat); Striatum; Dopamine D_2 receptor antagonist; Behavior

1. Introduction

Dopaminergic neurons project primarily to three regions in the brain: the striatal, mesocortical and tuberohypophysial regions (Björklund and Lindvall, 1984). These dopaminergic neurons are thought to be related to locomotion and motivation in animals (Kiyatkin et al., 1993; Wilson et al., 1994; Kiyatkin and Stein, 1995). In humans, lesions of the nigrostriatal dopamine neurons cause slowness of movement, such as that observed in Parkinson's disease (Horneykiewicz, 1966). Similar symptoms are observed in animals that are given the dopamine-specific neurotoxin, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), or its metabolite, 1-methyl-1,4-phenylpyridinium (MPP^+; Heikkila et al., 1984; Langston et al., 1984; Bradbury et al., 1986). Dopamine receptor antagonists also cause parkinsonism in humans, and these drugs induce cataleptic behavior in rodents (Fujiwara, 1992; Matsubara et al., 1993; Ossowska et al., 1993). Recently, studies using microdialysis have shown that the dopamine receptor antagonist, sulpiride, which induces cataleptic behavior, causes dopamine release in the striatum and subsequently elevated levels of the dopamine metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC; Zetterström et al., 1984; Imperato and Di Chiara, 1988). This raises the question of whether the mechanism underlying sulpiride-induced catalepsy is a direct effect of sulpiride itself on the cells or an indirect effect involving the newly synthesized and released dopamine or DOPAC. Zetterström et al. (1984) and Fujiwara (1992) concluded that increased dopamine or DOPAC is not related to catalepsy. However, these authors did not measure in vivo levels of sulpiride itself, and the relation between cataleptic behavior and extracellular intrastriatal sulpiride, dopamine and DOPAC concentrations was not investigated.

In the present study we first determined whether sulpiride was measurable in the brain using in vivo voltammetry and then examined the time-course of the increase in levels of extracellular sulpiride, dopamine and DOPAC in the striatum and compared this to the time-course of sulpiride-induced cataleptic behavior. A preliminary account of this work has been presented (Horikawa et al., 1995).

2. Materials and methods

2.1. Preparation of freely moving animals

The animals were prepared as described previously (Nakazato and Akiyama, 1988). Briefly, male Sprague-

* Corresponding author. Tel.: (81-25) 223-6161; Fax: (81-25) 225-6458; e-mail: hiroshi@bri.niigata-u.ac.jp

PII S0014-2999(97)00140-4

Dawley rats ($n = 10$, 350–450 g) were anesthetized with pentobarbital (50 mg/kg, intraperitoneal injection) and placed in a stereotaxic apparatus. A measuring electrode that consisted of a glass capillary microelectrode with a carbon fiber (7 μm in diameter) was inserted unilaterally into the right striatum (0.5 mm anterior and 3.0 mm lateral to bregma and 5.0 mm ventral to the dura mater). A reference electrode was placed on the dura mater and an auxiliary electrode was anchored to the skull. A stainless-steel cannula (0.6 mm outside diameter) was placed approximately 1 mm anterior to the working electrode on the same side and was inserted 2.5 mm into the brain just above the striatum at an oblique angle of 5° from vertical. The animals were allowed to recover for at least 4 weeks before use. The cannula was sealed with a dummy inner cannula until at least 30 min before use.

2.2. Electrochemical measurements

A cyclic voltammogram ranging from −400 to 1000 mV (25-mV steps) was used to determine the specific oxidation voltage of sulpiride (Fig. 1). The sulpiride current intensity was then measured as follows: the electrode was pretreated with an anodic-cathodic triangular wave (±1350 mV, 10 V/s slope), and 2 s after the wave, a triple-stepped pulse was applied (Fig. 2). The first step was to 650 mV for 660 ms, the second to 750 mV for 1160 ms, and the third to 850 mV for 162 ms. Dopamine and DOPAC current intensities were determined as described previously (Nakazato and Akiyama, 1992) with voltage steps from 150 mV to 250 mV and 250 mV to 350 mV, respectively. Homovanillic acid (HVA) current intensity was determined when the potential was stepped from 350 mV to 450 mV and MPP^+ current intensity was determined when the potential was stepped from 550 mV to 650 mV (Nakazato and Akiyama, 1993). The current intensity of each agent was measured every 3 min.

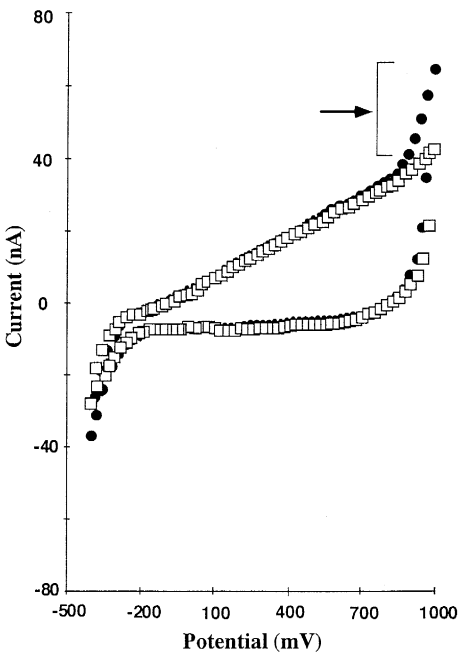

Fig. 1. Cyclic voltammogram in 10^{-4} M sulpiride (●). In the upper trace, sulpiride oxidative current indicated by the arrow was found at potentials over approximately 775 mV. The lower trace illustrates the measurements as the voltage was reduced from 1000 mV to −450 mV. Sulpiride was dissolved in PBS. In control trials (□), only PBS was used.

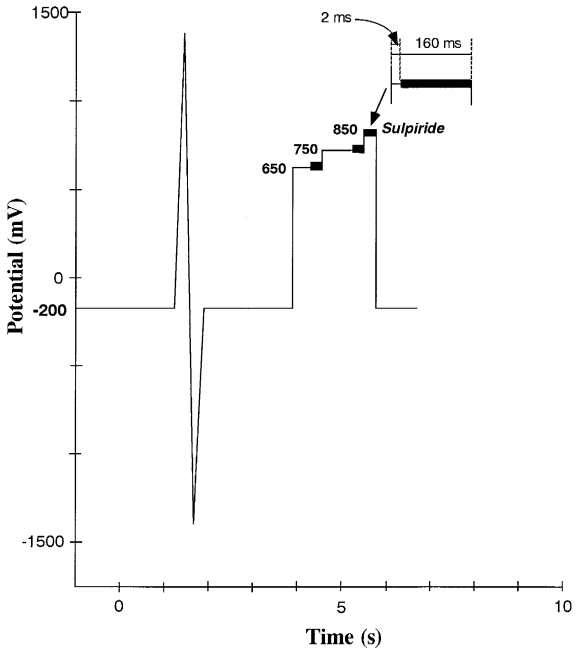

Fig. 2. Schematic representation of the potential profile used to measure the concentration of sulpiride. Current intensity was measured during the time indicated by the thick lines.

2.3. Drug administration

Sulpiride (Fujisawa Yakuhin Kogyo, Japan), dopamine (Sigma, St. Louis, MO, USA), HVA (Sigma), MPP^+ (Research Biochemicals International, Natick, MA, USA) and nomifensine (Research Biochemicals International) were dissolved at a concentration of 10^{-3} M in normal saline deaerated with nitrogen gas. All drugs were administered through a cannula positioned approximately 500–800 μm from a recording electrode. The inner stainless-steel cannula was made of a stainless-steel tube (0.3 mm outside diameter) into which a fused silica tube (0.15 mm outside diameter) had been threaded. The length of the inner cannula matched that of the outer cannula that had already been implanted in the brain. The fused silica tube protruded 2.5 mm from the tip of the cannula. The dummy inner cannula was removed and the inner cannula was inserted at least 30 min before the drug injection. A total of 7.5 μl of each drug was injected over a period of 30 min into the striatum by means of a microcomputer-controlled auto-injector.

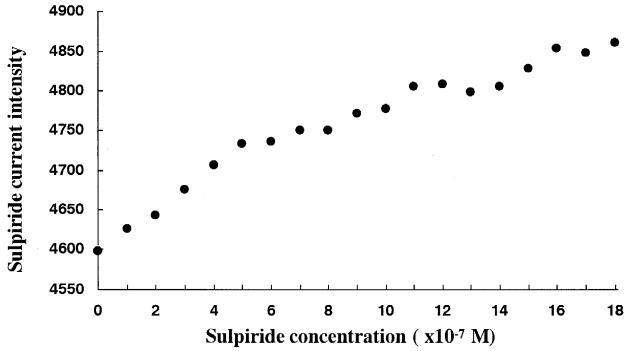

Fig. 3. Sulpiride current intensity as a function of sulpiride concentration in vitro.

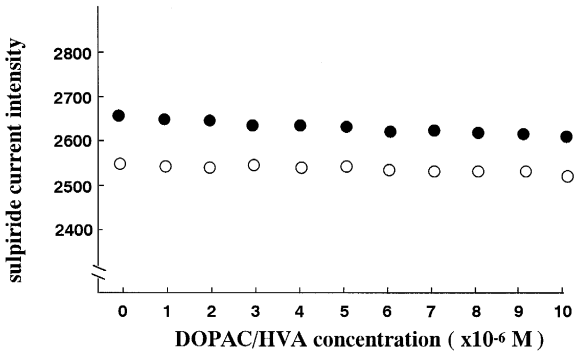

Fig. 5. Effects of DOPAC and HVA on sulpiride current intensity. Sulpiride current intensity was not influenced by the presence of DOPAC (○) or HVA (●).

2.4. Behavioral testing

Cataleptic behavior was evaluated and scored using a bar test. The animal was placed in a Plexiglas cage (25 × 30 × 25 cm) with a horizontal bar suspended 10 cm above the cage floor. The forepaws were gently placed on the bar and the time that the animal maintained this position was recorded. The scoring method was adopted from that of Imperato and Di Chiara (1985). Scores were assigned using a four-point scale: 0 = 0–14 s; 1 = 15–29 s; 2 = 30–59 s; and 3 = 60 s or more.

2.5. Histological procedure

After the in vivo experiments, the rats were perfused intracardially with phosphate-buffered saline (PBS) followed by 10% formalin. The brains were dissected out and cryo-sectioned into 50-μm-thick sections with a freezing microtome. The slices were mounted on poly-L-lysine-coated slides and stained with cresyl violet. The positions of the electrodes and cannula were confirmed by microscopy.

3. Results

3.1. Electrochemical measurements of sulpiride in vitro

At voltages over 775 mV, sulpiride oxidized and produced a measurable current (Fig. 1). When sulpiride was added in vitro, the sulpiride current intensity increased in a dose-dependent manner (Fig. 3). Concentrations of sulpiride in the ten nanomolar range could be detected. The DOPAC current intensity also increased in a dose-dependent manner (Fig. 4). DOPAC and HVA current intensities were not influenced by the presence of sulpiride (Fig. 5). In contrast, the current intensity of dopamine was affected by the presence of sulpiride, precluding accurate

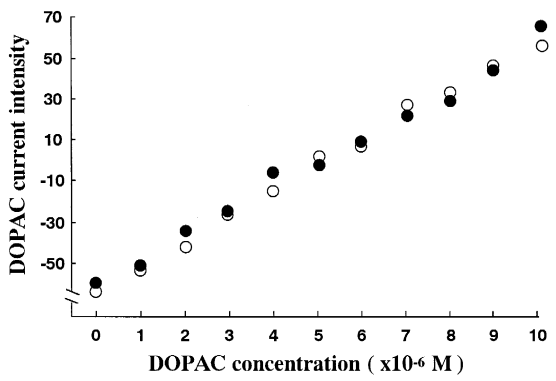

Fig. 4. DOPAC current intensity was not influenced by the presence of sulpiride. DOPAC concentration was increased from 10^{-6} M to 10^{-5} M in the presence of 10^{-4} M of sulpiride in PBS (●) or in PBS only (○).

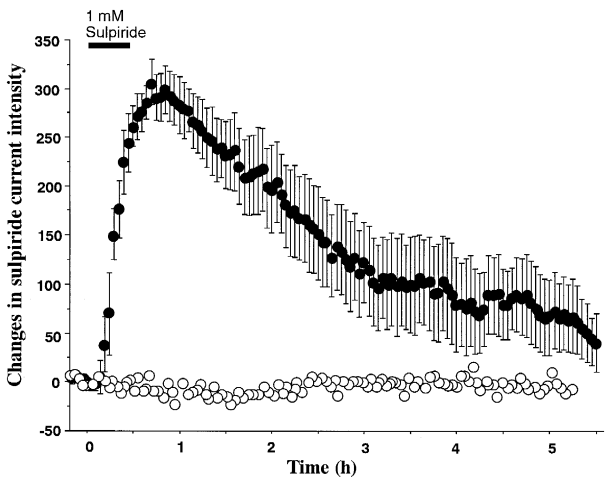

Fig. 6. Mean (±S.E.M.) changes in sulpiride current intensity when 10^{-3} M sulpiride (●) ($n = 5$), or normal saline as control (○) ($n = 5$), was intrastriatally administered during the time indicated by the bar. Measurements were normalized to the baseline current intensity just before the start of injection. Sulpiride remained in the extracellular fluid for over 4 h.

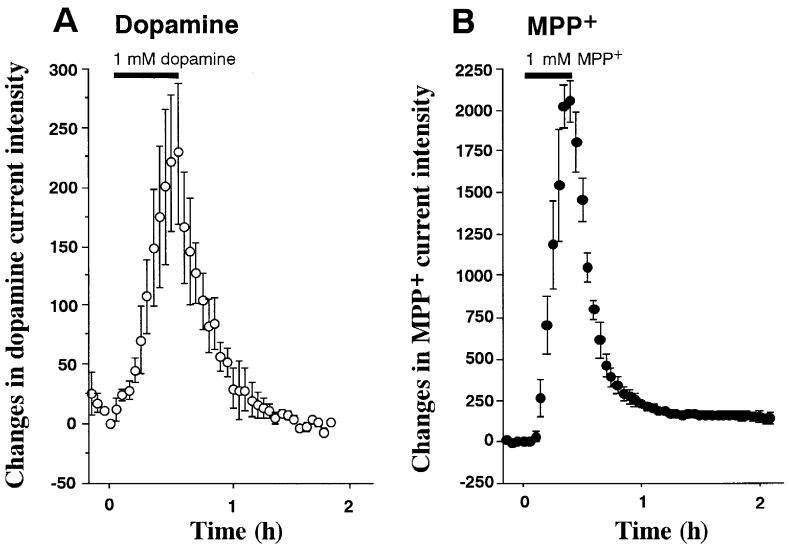

Fig. 7. Mean (±S.E.M.) changes of dopamine (A) and MPP^+ (B) current intensity when drugs were administered into the striatum at a concentration of 10^{-3} M. Drugs were injected over a 30 min period (indicated by the bar). Five animals were used for each drug.

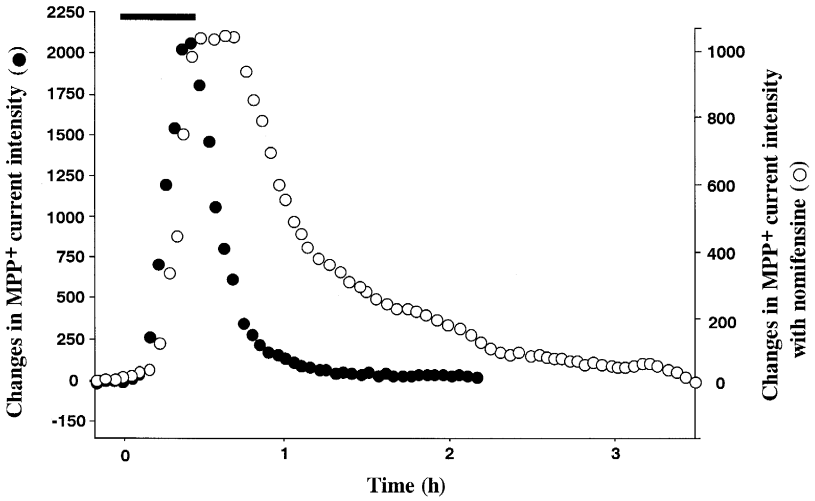

Fig. 8. Comparison of the time-course of the effect of MPP^+ alone and MPP^+ with nomifensine. Mean (±S.E.M.) changes of MPP^+ current intensity when MPP^+ (10^{-3} M) and nomifensine (2×10^{-3} M) were administered together into the striatum over a 30 min period (indicated by the bar). Three animals were used in the condition of MPP^+ and nomifensine, and five animals were in MPP^+ only.

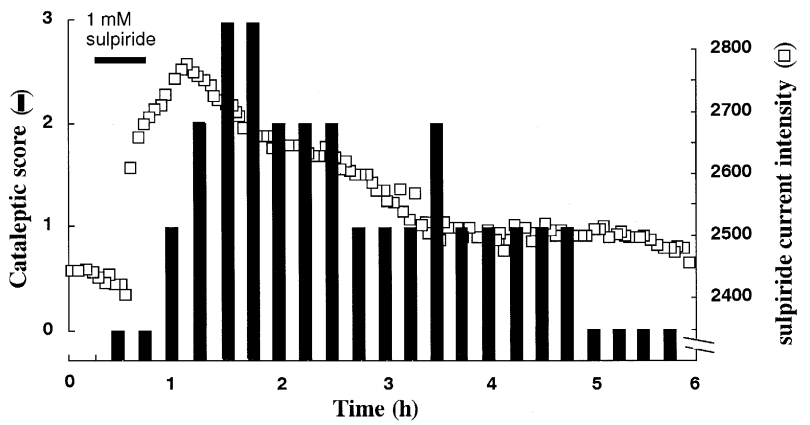

Fig. 9. The duration and intensity of cataleptic behavior paralleled the extracellular concentration of sulpiride. A representative case is shown. Behavioral scoring is detailed in the text.

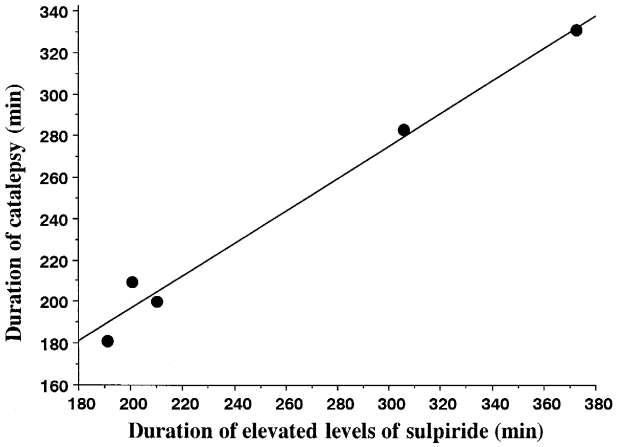

Fig. 10. Correlation between the duration of cataleptic behavior and the duration of elevated levels of sulpiride. The duration of cataleptic behavior (scores of 1–3) was measured from the end of drug administration. Duration of elevated levels of sulpiride was measured from the start of injection until sulpiride current intensity returned to its pre-injection value. $n = 5$, $r = 0.984$, $P < 0.001$.

measurement of the current intensity of dopamine when in the presence of sulpiride (data not shown).

3.2. Intrastriatal kinetics of sulpiride

When 7.5 μl of 10^{-3} M sulpiride was infused into the rat striatum over 30 min, the concentration of sulpiride reached a peak approximately 20 min (21 ± 3 min, $n = 5$) after the end of the injection, and returned to basal levels 3–5 h later (Fig. 6). The recovery time was significantly longer than that of intrastriatally administered dopamine or MPP^+ ($P < 0.005$ by Student's *t*-test for both comparisons), which returned to their basal levels within 1 h (Fig. 7A,B). When 10^{-3} M MPP^+ and its uptake blocker, nomifensine, were injected simultaneously into the striatum, the MPP^+ current intensity remained higher than the basal level for over 2 h ($n = 3$; Fig. 8). This recovery time

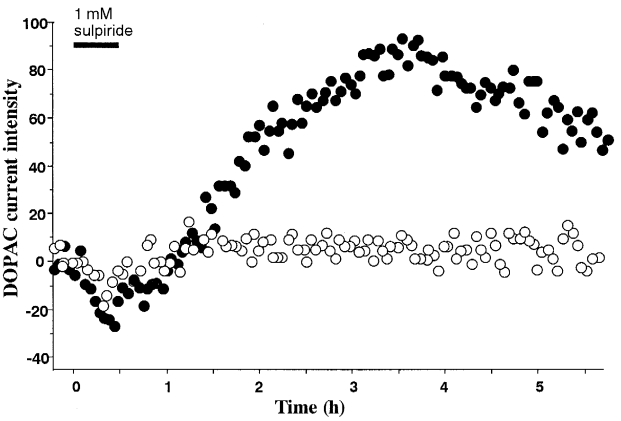

Fig. 11. Effect of 10^{-3} M sulpiride (●) on the extracellular DOPAC concentration in vivo. As a control, normal saline was given to the same rat (○). Sulpiride was injected during the time indicated by the bar.

was significantly longer ($P < 0.01$ by Student's *t*-test) than that of MPP^+ alone (< 1 h; $n = 5$).

3.3. Correlation between the extracellular concentration of sulpiride and catalepsy

Intrastriatal injection of 10^{-3} M sulpiride induced cataleptic behavior, the course of which paralleled the duration and magnitude of sulpiride concentration (Fig. 9). Catalepsy appeared approximately 45 min (45 ± 15 min) after the start of injection ($n = 5$), reaching a peak approximately 60–90 min after the end of the injection. The cataleptic behavior persisted until the sulpiride current intensity returned to its basal levels. As shown in Fig. 10, the duration of cataleptic behavior correlated well with that of the increase in sulpiride ($r = 0.984$, $P < 0.001$ by Pearson's correlation coefficient). In contrast, injection of normal saline alone did not cause behavioral change. The extracellular concentration of DOPAC was also measured while sulpiride was administered. The DOPAC concentration increased approximately 30 min after the end of injection, and reached a peak 180 min after the injection, but did not return to its basal levels within the experimental period (Fig. 11).

4. Discussion

In the present study, we examined the kinetics of intrastriatally administered sulpiride using in vivo voltammetry and compared the time-course of sulpiride concentration in the extracellular space with the time-course of the cataleptic behavior induced by the injection of sulpiride. First, using an in vitro preparation, we ascertained that sulpiride was measurable using this method and that sulpiride had a unique oxidation/reduction potential, making it possible to differentiate it from dopamine, DOPAC and HVA. We then extended these in vitro experiments to determine the time-course of sulpiride concentration in vivo. The extracellular concentration of sulpiride increased and remained elevated for a long period after intrastriatal injection. In contrast, the extracellular concentration of dopamine, DOPAC and MPP^+ decreased immediately after the end of the injection (Nakazato and Akiyama, 1988, 1993; Cass et al., 1993). This result introduces the question of why the administered sulpiride peaked later than dopamine or MPP^+ and remained longer in the extracellular fluid. Three major processes by which compounds are cleared from the extracellular space are diffusion, metabolism and uptake into nearby cells. It is known that dopamine and MPP^+, but not sulpiride, can be taken up via dopamine transporter proteins (Shimada et al., 1991; Cass et al., 1993; Nakazato and Akiyama, 1993; Valchar and Hanbauer, 1993). In addition, sulpiride is not metabolized (Bressolle et al., 1984). Because sulpiride is not

metabolized or transported, it accumulates up to higher concentrations than dopamine, thus taking longer to reach its peak concentration (20 min vs. 6 min). Furthermore, the present results showed that when MPP^+ was administered simultaneously with the uptake inhibitor nomifensine, MPP^+ peaked later and remained in the extracellular fluid longer than when MPP^+ was administered alone. Thus, the level of sulpiride was most likely maintained because it is neither metabolized nor taken up by neurons or glial cells.

The results of the present experiments showed that sulpiride-induced catalepsy in rats generally paralleled the increase in extracellular sulpiride, with a slight delay in onset. Catalepsy appeared approximately 30 min later than the increase in sulpiride concentration. This delay in the onset of catalepsy may be due to diffusion of the injected sulpiride; although the sulpiride concentration increased soon after the injection immediately around the injection site, sulpiride may have continued to diffuse away until a sufficiently wide area was affected to produce the behavioral effect. The results confirm that sulpiride causes increases in extracellular DOPAC, indicating that sulpiride also induced dopamine release. In contrast to those of sulpiride, however, the DOPAC levels remained elevated after cataleptic behavior disappeared, indicating that dopamine levels also remained elevated after behavior had returned to normal. These results support a direct effect of sulpiride itself on the induction of cataleptic behavior, and not of the related increased extracellular levels of dopamine or DOPAC.

The monoaminergic systems have important roles in various behavioral functions, and their dysfunction is thought to cause many neurological and psychological disorders, such as schizophrenia, Parkinson's disease and depression (Bunney and Davis, 1965; Horneykiewicz, 1966; Brown and Linnoila, 1990; Davis et al., 1991; Seeman, 1992). Many dopamine receptor agonists and antagonists are known to be effective in such disorders. In clinical studies, however, the exact relationships between the extracellular concentration of these drugs in the brain and the pharmacological effects are, for the most part, unknown. Results of the present study, together with those of previous studies, indicate that some of these drugs, such as sulpiride, may remain in the brain for unusually long periods and may lead to secondary changes of intrinsic dopaminergic regulation. It is important, therefore, to re-examine the pharmacokinetics of exogenous and endogenous dopaminergic substances. This may aid in the development of dopaminergic agents with more specific therapeutic benefit and fewer side effects.

Acknowledgements

We thank Emeritus Professor Mei Satake (Department of Neurochemistry, Niigata University) and Professor Hiroyuki Nawa (Department of Molecular Neurobiology, Niigata University) for helpful discussions. This study was supported in part by grants (to H.P.M.H.) from Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

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