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EFFECTS OF MIDAZOLAM AND FLUNITRAZEPAM ON THE RELEASE OF DOPAMINE FROM RAT STRIATUM MEASURED BY *IN VIVO* MICRODIALYSIS

K. TAKADA, T. MURAI, T. KANAYAMA AND N. KOSHIKAWA

SUMMARY

We have studied the effects of midazolam and flunitrazepam on extracellular concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in rat striatum in freely moving animals using in vivo *microdialysis. I.v. injections of midazolam 0.075 and 0.15 mg kg^{-1} decreased striatal dopamine concentrations in a dose-dependent manner without affecting the concentrations of DOPAC and HVA. Flunitrazepam 0.015 and 0.03 mg kg^{-1} also decreased striatal dopamine concentrations in a dose-related manner, but the reductions in DOPAC and HVA were not significant. Flumazenil 6 μg kg^{-1} alone did not affect striatal concentrations of dopamine, DOPAC and HVA, but it prevented the effects of midazolam and flunitrazepam. Flunitrazepam 10 μmol litre^{-1} also decreased striatal dopamine release when infused through a dialysis probe placed into the striatum, but it failed to affect striatal dopamine release when infused into the ipsilateral substantia nigra. Central administrations of midazolam were effective only when the drug was infused into both sites simultaneously (10 and 100 μmol litre^{-1}) or given by intraventricular injection (0.5 and 1 μg). These results suggest that midazolam and flunitrazepam affect striatal dopamine release in a different manner.* (Br. J. Anaesth. 1993; **70**: 181–185)

KEY WORDS

Brain: striatal catecholamines. Hypnotics, benzodiazepines flunitrazepam, midazolam. Sympathetic nervous system: dopamine.

Behavioural and biochemical studies suggest that there is a functional interaction between γ-aminobutyric acid (GABA)-ergic and dopaminergic neuronal systems, especially at dopaminergic nerve terminals [1–5]. For example, GABA receptor agonists injected into the substantia nigra have been reported to reduce striatal dopamine release [6, 7]. With the brain microdialysis technique, which permits measurement of dopamine and its metabolites in discrete brain regions *in vivo*, it has also been shown that dopamine release in the striatum and nucleus accumbens is decreased by benzodiazepines administered systemically or by local intracerebral injection [8–10]. The actions of the benzodiazepines are caused by enhancement of the action of GABA and are mediated by specific binding sites which are part of the multimolecular $GABA_A$–receptor complex [10–13].

Benzodiazepines are given i.v. to produce either sedation or, when combined with an opioid analgesic, anaesthesia. Midazolam is the drug of choice for these purposes because it does not produce local venous irritation and thrombosis. However, there is still no clear understanding of its mode of action in the central nervous system.

The present study describes the effects of midazolam and of flunitrazepam, in doses similar to those used clinically, on nigrostriatal dopaminergic neuronal activity by using *in vivo* brain microdialysis to measure striatal dopamine release. As the striatum is known to be a terminal area of the dopaminergic neurones that arise from the substantia nigra [14], the compounds were also applied focally to the substantia nigra and the striatum to determine if there were differences between the sites of action of the two drugs.

MATERIALS AND METHODS

Male Sprague–Dawley rats (250–300 g body weight) were studied. Between experiments they were housed in a temperature-controlled environment on a 12-h light–dark cycle (light period 07:00–19:00) with free access to food and water.

Surgery

The rats were anaesthetized with pentobarbitone 50 mg kg^{-1} i.p. The left external jugular vein was cannulated and connected to an osmotic minipump which was filled with heparin–saline 1000 u ml^{-1} and implanted s.c. between the scapulae. The anaesthetized animals were placed in a stereotactic apparatus and unilateral guide cannulae were implanted just above the striatum (antero–posterior (AP) 9.2 mm, medio–lateral (ML) 3.0 mm, dorso–ventral (DV) 7.0 mm) and substantia nigra (AP 3.7 mm, ML 2.0 mm, DV 4.0 mm) according to

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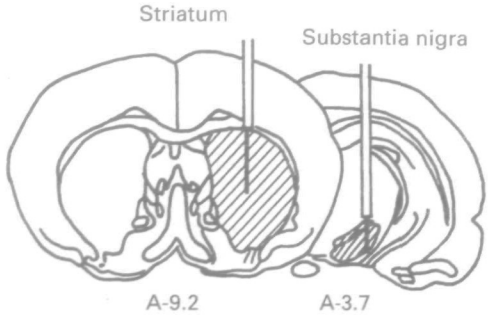

FIG. 1. Schematic illustration showing the location of the probe in the striatum and substantia nigra. Planes are taken from the atlas of Paxinos and Watson [15]; co-ordinates are in mm anterior to the interaural line.

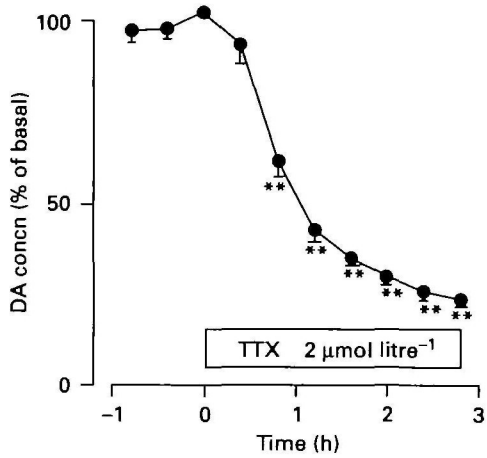

FIG. 2. Effects of infusion of tetrodotoxin (TTX) 2 μmol litre^{-1} on the concentrations of dopamine (DA) in striatal dialysates (mean, SEM of data from six rats). $^{**}P < 0.01$ compared with value just before TTX infusion (paired t test).

the atlas of Paxinos and Watson [15] (fig. 1). The guide cannulae were kept patent between experiments by stainless steel inserts. Before an experiment, the insert was removed and a dialysis probe (3 mm length for the striatum and 2 mm for the substantia nigra, 0.25 mm o.d., 50000 mol. wt "cut-off") was inserted into the guide cannulae so that only the dialysis tubing protruded from the tip. In some animals, a unilateral cannula (0.6 mm o.d., i.d. 0.3 mm) was implanted into the contralateral lateral ventricle (AP 8.2 mm, ML 1.5 mm, DV 7.0 mm) for intraventricular drug injections. The rats were then allowed to recover for a minimum of 7 days before experiments were carried out.

Dialysis and neurochemical measurements

On the day of the experiment, the probes were inserted carefully into the conscious rat and fixed to the guide cannulae by either a screw or dental cement. The rat was then placed in a plexiglass box (30 × 30 cm) and the inlet and outlet tubes connected to a swivel located on a counterbalanced beam to minimize discomfort to the rat. The probes were perfused at a rate of 2.0 μl min^{-1} with Ringer solution (composition (mmol litre^{-1}); NaCl 147, KCl 4, $CaCl_2$ 1.2, $MgCl_2$ 1.1; pH 6.0) and the outflow was connected by Teflon tubing to an HPLC system (EICOM, Kyoto, Japan). Perfusate samples were taken every 25 min for measurement of dopamine, DOPAC and HVA. When the baseline concentration of dopamine had stabilized (at least 4 h after probe insertion), drugs were injected via a jugular cannula. Baseline values were the mean of the last three samples before the injection of drug or infusion of tetrodotoxin. The probes had an *in vitro* recovery of 10–12% for dopamine, DOPAC and HVA, but the reported concentrations were not adjusted for recovery because this cannot be estimated accurately [16, 17].

Dopamine, DOPAC and HVA were separated on an Eicompak MA-5ODS column (5 μm, 4.6 × 250 mm, Eicom) using citrate buffer 0.07 mol litre^{-1} with octane-sulphonic acid 1.5 mmol litre^{-1}, EDTA 30 μmol litre^{-1}, 15% methanol (pH 3.9) as the mobile phase at a flow rate of 1.0 ml min^{-1}. The compounds were measured by electrochemical detection using a glassy carbon working electrode set at +700 mV against a silver–silver chloride reference electrode. The detection limit for each of the compounds was about 1 pg per sample. In some experiments, dopamine was separated on an Eicompak CA-5ODS column (5 μm, 4.6 × 250 mm, Eicom) using phosphate buffer 0.1 mol litre^{-1} with octane-sulphonic acid 5.8 mmol litre^{-1}, EDTA 0.3 mmol litre^{-1}, 20% methanol (pH 6.0) as a mobile phase (flow rate 1.0 ml min^{-1}). The working electrode was set at +400 mV against a silver–silver chloride reference electrode and this gave a detection limit for dopamine of about 500 fg per sample.

Drugs

Drugs used were midazolam (Dormicum, Yamanouchi Pharmaceutical Co., Ltd), flunitrazepam (Rohypnol, Nippon Roche), flumazenil (Ro 15-1788, Hoffmann-La Roche), sulpiride (Dogmatyl, Fujisawa Pharmaceutical Co., Ltd) and tetrodotoxin (Sigma). Drugs were diluted in 0.9% saline solution for i.v. injections, in Ringer solution for intraventricular injections or added to the perfusion medium for 20 min for intracerebral administration.

Histology

At the end of an experiment, the rat was anaesthetized deeply with pentobarbitone and perfused transcardially with 10% formalin. The brains were removed, sectioned (50 μm) and stained with cresyl violet to facilitate probe location.

Statistical analysis

All values were expressed as a percentage of baseline and analysed using either paired t test or two-way analysis of variance (ANOVA), as appropriate. Statistical significance was assumed at $P < 0.05$.

RESULTS

The basal concentrations of dopamine, DOPAC and HVA in the dialysate were 9.0 (SEM 1.8) pg/20 min ($n = 6$), 622 (124) pg/20 min and 405 (92) pg/20 min, respectively. The concentrations required 4 h to stabilize after probe insertion, but were then unaffected by saline 1 ml i.v. and remained stable for at least a further 4 h (data not shown). Tetrodotoxin

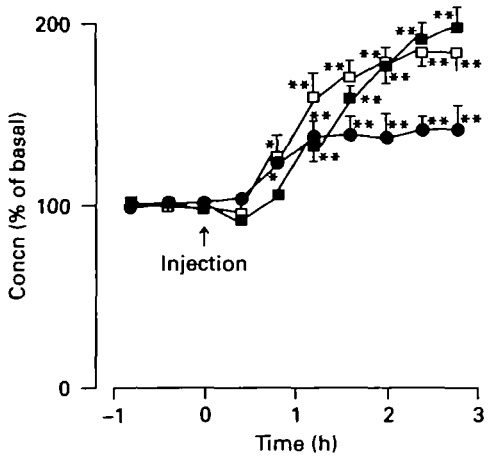

FIG. 3. Effects of i.v. injection of sulpiride 10 mg kg^{-1} on the concentrations of dopamine (●), DOPAC (□) and HVA (■) in striatal dialysates (mean, SEM of data from six rats). *$P < 0.05$; **$P < 0.01$ compared with value just before administration of sulpiride (paired t test).

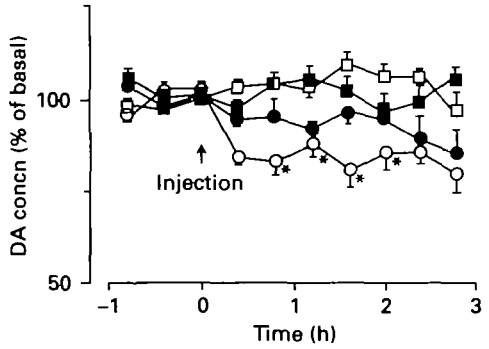

FIG. 4. Effects of midazolam and flumazenil on the concentrations of dopamine (DA) in striatal dialysates (mean, SEM of data from six rats). ● = Midazolam 0.075 mg kg^{-1}; ○ = midazolam 0.15 mg kg^{-1}; □ = flumazenil 6 μg kg^{-1}; ■ = midazolam 0.15 mg kg^{-1} + flumazenil 6 μg kg^{-1}. *$P < 0.05$ compared with value just before administration of drugs (paired t test).

2 μmol litre^{-1} infused into the striatum via the dialysis probe reduced basal concentrations of dopamine to approximately 20% of control (fig. 2).

Sulpiride 10 mg kg^{-1} i.v. increased concentrations of dopamine, DOPAC and HVA in the striatal dialysate. Their values at 3 h after injection were approximately 140%, 180% and 200% of control, respectively (fig. 3).

Midazolam 0.075 mg kg^{-1} and 0.15 mg kg^{-1} i.v. decreased concentrations of dopamine in striatal dialysates in a dose-dependent manner (fig. 4); the overall reductions during a 3-h observation period were 9% and 17%, respectively. However, midazolam did not significantly alter the concentrations of DOPAC and HVA (data not shown). The effects of midazolam 0.15 mg kg^{-1} were prevented by co-administration of flumazenil 6 μg kg^{-1}, a benzodiazepine receptor antagonist ($P < 0.01$, two-way ANOVA). Flumazenil alone did not affect concentrations of dopamine, DOPAC and HVA in striatal dialysates. Flunitrazepam 0.15 mg kg^{-1} and 0.03 mg kg^{-1} i.v. also decreased the concentrations of dopamine in striatal dialysates in a dose-related manner (fig. 5); the overall reductions during a 3-h observation period were 8% (0.015 mg kg^{-1}) and 24% (0.03 mg kg^{-1}), and tended to decrease concentrations of DOPAC and HVA (data not shown). The peak effects occurred approximately 100 min after injection of flunitrazepam (fig. 5) and were prevented ($P < 0.01$, two-way ANOVA) by co-administration of flumazenil 6 μg kg^{-1}.

A 20-min infusion of flunitrazepam 10 μmol litre^{-1} into the striatum via the dialysis probe significantly reduced (26% at peak) dialysate concentrations of dopamine for more than 1 h, but similar infusions of midazolam (10 and 100 μmol litre^{-1}) were ineffective (fig. 6); midazolam 1 mmol litre^{-1} was also ineffective (data not shown). Neither compound affect striatal dopamine release when infused into the ipsilateral substantia nigra, via a dialysis probe, in a concentration of 100 μmol litre^{-1} (fig. 6). However, midazolam 10 and 100 μmol litre^{-1} infused simultaneously into both sites (striatum and the ipsilateral substantia nigra) or injected into the lateral ventricle (0.5 μg and 1 μg in 5 μl) reduced the concentrations of dopamine in striatal perfusates in a dose-dependent manner (fig.7). The peak reductions in dopamine concentration after simultaneous infusion of midazolam into both the striatum and substantia nigra were 14% (10 μmol litre^{-1}) and 27% (100 μmol litre^{-1}); and peak reductions after injection of midazolam into the lateral ventricle were 14% (0.5 μg) and 22% (1 μg).

DISCUSSION

The sodium channel blocker, tetrodotoxin has been used extensively in brain microdialysis experiments to demonstrate that the neurotransmitter and its metabolites detected in the perfusates have been released by action potential-induced depolarization and not released passively as a result of local tissue damage [18]. In the present study, approximately 80% of the dopamine release was prevented by tetrodotoxin, indicating that it was caused mainly by neural activity.

I.v. midazolam and flunitrazepam decreased striatal dopamine release in a dose-dependent manner, in agreement with an earlier diazepam study [8]. Flunitrazepam was more effective than midazolam in doses similar to those used clinically to produce i.v. sedation and anaesthesia [19–21]. Concentrations of metabolites (DOPAC and HVA) in striatal dialysates tended to decrease after flunitrazepam, but were not affected by i.v. administration of midazolam. This lack of effect on the metabolites can be explained by the fact that the basal extracellular concentrations of DOPAC and HVA were 70 and 45 times greater than the concentration of dopamine; thus it can be assumed that a small (about 20%) reduction in dopamine release induced by midazolam and flunitrazepam might not be sufficient to change the concentrations of the metabolites. The lack of effect, however, could not be caused by the methodological difficulties in measuring the metabolites as sulpiride, a drug reported to increase the extracellular concentration of dopamine, DOPAC and HVA by block of dopaminergic D_2 autoreceptors, increased the concentrations of these compounds to values similar

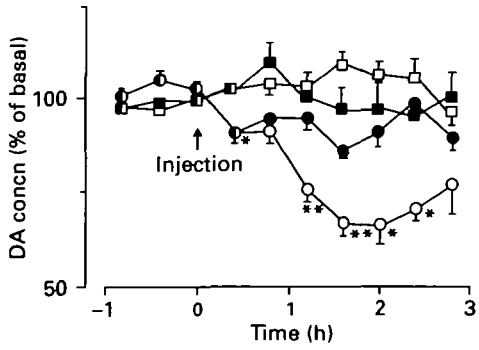

FIG. 5. Effects of flunitrazepam and flumazenil on the concentrations of dopamine (DA) in striatal dialysates (mean, SEM of data from six rats). ● = Flunitrazepam 0.015 mg kg^{-1}; ○ = flunitrazepam 0.03 mg kg^{-1}; □ = flumazenil 6 μg kg^{-1}; ■ = flunitrazepam 0.03 mg kg^{-1} + flumazenil 6 μg kg^{-1}. *$P < 0.05$, **$P < 0.01$ compared with value just before administration of drugs (paired t test).

to those reported previously [22]. The findings of the present study with midazolam and flunitrazepam suggest that, in the dose range used clinically, flunitrazepam had more pronounced effects on dopaminergic pathways than midazolam. These effects of midazolam and flunitrazepam were mediated by the $GABA_A$–benzodiazepine receptor complex, which inhibits the dopaminergic neuronal activity, because they were abolished by flumazenil.

As the striatum is known to be a terminal area of the dopaminergic neurones that arise from the substantia nigra, and their activities are controlled by the GABAergic inhibitory neurones, we were interested in examining the brain site responsible for the ability of the benzodiazepines to reduce the striatal dopamine release. To determine if systemically administered midazolam and flunitrazepam reduced striatal dopamine release by action in the terminal field (striatum) or in the cell body region (substantia nigra) of the nigrostriatal dopaminergic neurones, the compounds were applied directly to these structures by infusion through dialysis probes. Small concentrations of flunitrazepam infused into the striatum were effective, whereas larger concentrations infused into the substantia nigra were ineffective. This suggests that flunitrazepam reduced dopamine release by action at $GABA_A$–benzodiazepine receptors in the terminal field of the dopaminergic pathways. For midazolam to reduce striatal dopamine release, it was necessary to stimulate $GABA_A$–benzodiazepine receptors in both the terminal and cell body regions of the dopaminergic neurones by infusing it into both the striatum and substantia nigra simultaneously or by giving it intraventricularly. The effects of midazolam and flunitrazepam were antagonized by flumazenil, but this does not exclude the possibility that the two compounds were acting on different receptor subtypes [23], because flumazenil is not selective. Regional differences in the effects of benzodiazepines on dopamine release have been shown previously

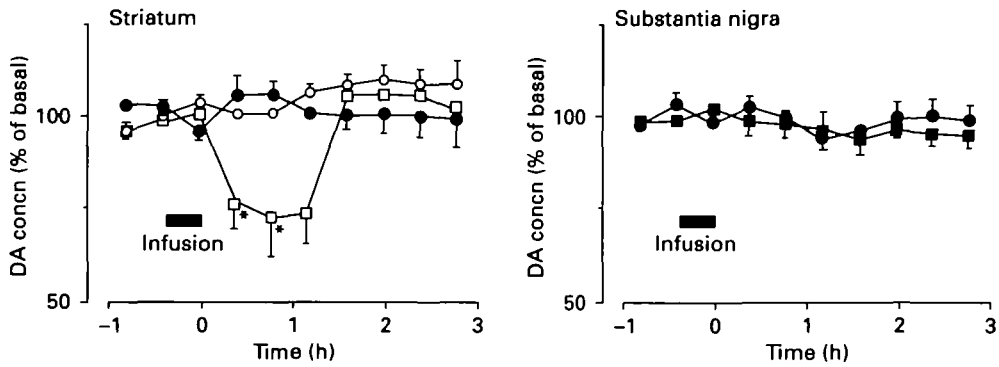

FIG. 6. Effects of infusion of midazolam 10 μmol litre^{-1} (○) or 100 μmol litre^{-1} (●) and flunitrazepam 10 μmol litre^{-1} (□) or 100 μmol litre^{-1} (■) into either the striatum (left) or ipsilateral substantia nigra (right) on concentrations of dopamine (DA) in striatal dialysates (mean, SEM of data from six rats). *$P < 0.05$ compared with value just before administration of drugs (paired t test).

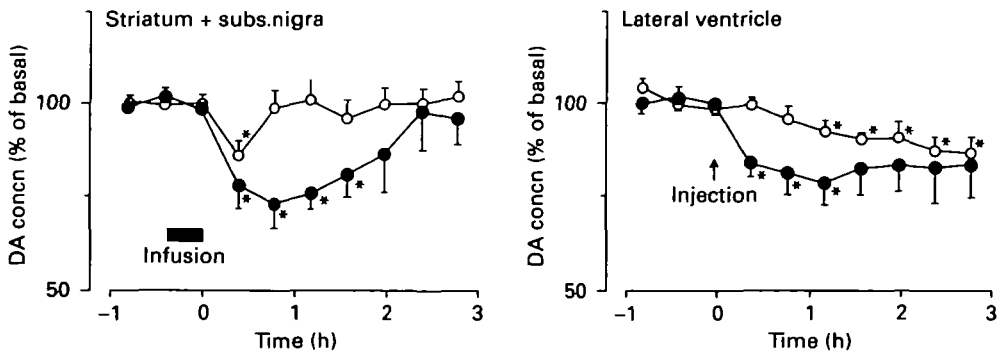

FIG. 7. Effects of infusion of midazolam 10 μmol litre^{-1} (○) or 100 μmol litre^{-1} (●) into both the striatum and ipsilateral substantia nigra (left) and injection of midazolam 0.5 μg 5 μl^{-1} (○) or 1.0 μg 5 μl^{-1} (●) into the lateral ventricle (right) on the concentrations of dopamine (DA) in striatal dialysates (mean, SEM of data from six rats). *$P < 0.05$ compared with value just before administration of midazolam (paired t test).

with systemic and central injections of diazepam and flurazepam which reduced dopamine release in the nucleus accumbens more than in the striatum [8, 10]. These regional differences can be explained by assuming a reduced basal GABA tone or more efficient receptor coupling in the nucleus accumbens and, therefore, more scope for a benzodiazepine effect in the structure. These two possibilities (regional differences in GABA tone and receptor coupling) may also explain the regional differences between the effects of midazolam and flunitrazepam observed in the present study.

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