Pharmacology Biochemistry and Behavior, Vol. 60, No. 3, pp. 695–701, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00050-1

Clonidine Reduces Dopamine and Increases GABA in the Nucleus Accumbens: An In Vivo Microdialysis Study

TAMEATSU MURAI,* YUKIHIRO YOSHIDA,* SHIGEYO KOIDE,* KOJI TAKADA,* TORU MISAKI,* NORIAKI KOSHIKAWA† AND ALEXANDER R. COOLS‡

*Departments of *Dental Anesthesiology and †Pharmacology, Nihon University School of Dentistry, 1-8-13, Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8310, Japan*
‡Department of Psychoneuropharmacology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

Received 5 September 1997; Revised 16 December 1997; Accepted 30 December 1997

MURAI, T., Y. YOSHIDA, S. KOIDE, K. TAKADA, T. MISAKI, N. KOSHIKAWA AND A. R. COOLS. *Clonidine reduces dopamine and increased GABA levels in the nucleus accumbens: An in vivo microdialysis study.* PHARMACOL BIOCHEM BEHAV **60**(3) 695–701, 1998.—The effects of clonidine, an α_2 adrenoceptor agonist, on extracellular concentrations of dopamine and γ-aminobutyric acid (GABA) in the nucleus accumbens of rats were studied by using in vivo brain microdialysis. Clonidine (5 μg/kg IV) significantly decreased the brain microdialysate concentration of dopamine in the nucleus accumbens up to a maximum of 16% at its peak effect. This effect was inhibited by a dose of idazoxan (10 μg/kg IV), an α_2-adrenoceptor antagonist, which itself did not affect the efflux of dopamine. A smaller dose of clonidine (1 μg/kg IV), which had no significant effect on dopamine efflux per se, decreased the dopamine efflux (21% reduction) when given together with an ineffective dose of midazolam (0.075 mg/kg IV), a benzodiazepine receptor agonist. The effect of clonidine (5 μg/kg IV) on mesolimbic dopamine efflux was abolished by bicuculline (1 mg/kg IV), a $GABA_A$ receptor antagonist, counteracted by β-carboline-3-carboxylate ethyl ester (β-CCE, 3 mg/kg IP), a benzodiazepine receptor inverse agonist, but not affected by flumazenil (6 μg/kg IV), a benzodiazepine receptor antagonist. Clonidine (5 μg/kg IV) increased the dialysate concentration of GABA in the nucleus accumbens up to a maximum of 250% at its peak effect, but not in the ventral tegmental area. It is hypothesized that $GABA_A$ binding sites in the nucleus accumbens form part of the sequence of events that is triggered by clonidine in an α_2-adrenergic-specific manner and that ultimately results in a decreased release of dopamine in the nucleus accumbens. ©1998 Elsevier Science Inc.

Clonidine Midazolam Nucleus accumbens Dopamine release GABA release

BENZODIAZEPINES are frequently used as a preoperative medication because of their sedative, anxiolytic, and anterograde amnestic properties (13). Addition of aminergic drugs has been found to significantly alter the anesthetic requirements for benzodiazepines. Thus, clonidine and dexmedetomidine, two highly selective, specific and potent α_2-adrenoceptor agonists, decrease the requirements for benzodiazepines (9,19,25,26), whereas cocaine and dexamphetamine, namely drugs that among others stimulate the release of dopamine, enhance the requirements for anesthetics (11,29). Because low doses of cocaine and dexamphetamine release dopamine preferentially in the nucleus accumbens (7), this nucleus may play an important role in the mutual interaction between aminergic drugs and benzodiazepines. Indeed, it has been repeatedly shown that benzodiazepines preferentially decrease the release of dopamine in the nucleus accumbens (8,10,33). Moreover, compounds such as reserpine, which deplete dopamine stores present in mesolimbic dopaminergic terminals, decrease the requirements for anesthetics (12). This may at least partially explain the synergistic interaction between reserpine and anesthetics.

The reduction in the release of mesolimbic dopamine that is produced by benzodiazepines is mediated by the γ-aminobutyric acid $(GABA)_A$/benzodiazepine receptor complex (18). Thus, the effects of benzodiazepines upon dopamine release in

Requests for reprints should be addressed to Dr. Noriaki Koshikawa, Department of Pharmacology, Nihon University School of Dentistry, 1-8-13, Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8310, Japan.

the nucleus accumbens are indirect. However, it is not yet known whether the $GABA_A$/benzodiazepine complex that modulates the release of mesolimbic dopamine, belongs to GABAergic neurons that terminate within the nucleus accumbens or to striatonigral, GABAergic neurons that impinge upon dopaminergic neurons terminating in the nucleus accumbens.

As mentioned above, α_2-adrenoceptor agonists also decrease the anesthetic requirements for benzodiazepines (9,19,25). Although it is presumed that this reduction is caused by actions at both pre- and postsynaptic α_2-adrenoceptors in the central nervous system, the exact site of action is not yet known. However, the synergistic interaction between adrenergic compounds and benzodiazepines is certainly not due to a pharmacodynamic interaction at the level of their receptors (25). Given the abovementioned role of the nucleus accumbens in the interaction between dopamine and anesthetics, it is important to note that the nucleus accumbens also contains α_2-adrenoceptors (32). Moreover, there is biochemical and behavioural evidence that mesolimbic noradrenaline can inhibit the dopaminergic activity in the nucleus accumbens (6,20). Furthermore, α_2-adrenoceptors are located on adrenergic cell bodies of neurons that innervate the nucleus accumbens (32). In other words, there are at least two sites of action that may allow α_2-adrenergic compounds to modulate mesolimbic dopamine.

Therefore, the first aim of this study was to investigate whether the α_2-adrenoceptor agonist clonidine can modulate the release of dopamine in the nucleus accumbens in an adrenergic-specific manner. The second aim of this study was to determine whether benzodiazepines are involved in the mechanism by which clonidine alters nucleus accumbens dopamine. For that purpose, it was investigated whether clonidine can modulate the effects of midazolam upon the dopamine release in the nucleus accumbens. The final aim of this study was to investigate whether the $GABA_A$/benzodiazepine complex plays a role in the effects of clonidine upon the dopaminergic activity in the nucleus accumbens. For that purpose, two sets of experiments were performed. First, it was analyzed to which extent drugs that selectively affect the $GABA_A$/benzodiazepine complex change the effects of clonidine upon the release of mesolimbic dopamine. Given the outcome of this experiment, it was decided to investigate whether clonidine alters mesolimbic, dopamine release via GABAergic neurons that terminate within this nucleus or via GABAergic neurons that impinge upon dopaminergic cells that project to the nucleus accumbens. For that purpose, the ability of clonidine to alter GABA release in the nucleus accumbens and the ventral tegmental area was assessed.

The present study shows that clonidine decreases the release of dopamine in the nucleus accumbens in an adrenergic-specific manner and via a $GABA_A$/benzodiazepine complex. It is suggested that this complex belongs to GABAergic neurons that terminate in this nucleus.

METHOD

Animals

Male Sprague–Dawley rats (250–300 g body weight) were used. They were housed in a temperature-controlled environment on a 12 L:12 D cycle (light period 0700–1900 h) with free access to food and water.

Surgery

The rats were anesthetized with sodium pentobarbitone (50 mg/kg IP). The left external jugular vein was cannulated and connected with Teflon tubing to an osmotic minipump, filled with heparin-saline (1000 U/ml), implanted subcutaneously between the scapulae. The Teflon tubing was exteriorized to permit intravenous drug injections. The anesthetized animals were placed in a stereotactic apparatus and unilateral guide cannulae were implanted just above the nucleus accumbens (anteroposterior (AP) 10.6 mm, mediolateral (ML) 1.5 mm, dorsoventral (DV) 4.0 mm from interaural line), and the ventral tegmental area (AP 4.2 mm, ML 1.0 mm, DV 2.0 mm) according to the atlas of Paxinos and Watson (21) (Fig. 1). To avoid the ventricular system, the cannulae directed at the nucleus accumbens were angled 18 degrees from the midsagittal plane and those directed at the ventral tegmental area were angled 20 degrees from that plane. After completing surgery, the rats were allowed to recover for a minimum of 7 days before experiments were carried out, the guide cannulae being kept patent by stainless steel inserts. In this experiment each animal was used for one experimental group and was used only once. All experiments were performed according to institutional and national guideline of animal experimentation.

Dialysis and Neurochemical Measurements

The commercially available I-shaped removable-type dialysis probe (2 mm length regenerated cellulose membrane, 0.25 mm o.d., 50,000 mol. wt. "cutoff," EICOM A-I-8-02 type, Kyoto, Japan) was used throughout the experiments. The stylett was removed from the guide cannula before each experiment and the dialysis probe inserted so that only the dialysis tubing protruded from the tip. The probe was secured to the guide cannula by a screw. The rat was then placed in a Plexiglas 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 probe was perfused at a rate of 2.0 μl/min with modified Ringer solution (NaCl 147 mM, KCl 4 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.1 mM; pH 6.0) and the outflow connected by Teflon tubing to a high-performance liquid chromatography system (EICOM, Kyoto, Japan). Dopamine efflux stabilized 4 h after probe insertion, at which time it was substantially reduced by the sodium channel blocker, tetrodotoxin, demonstrating that release was occurring mainly as a result of neural activity (18,30). For GABA efflux, it was necessary to stabilize the GABA content in the perfusate for 24 h after probe insertion. Perfusate samples

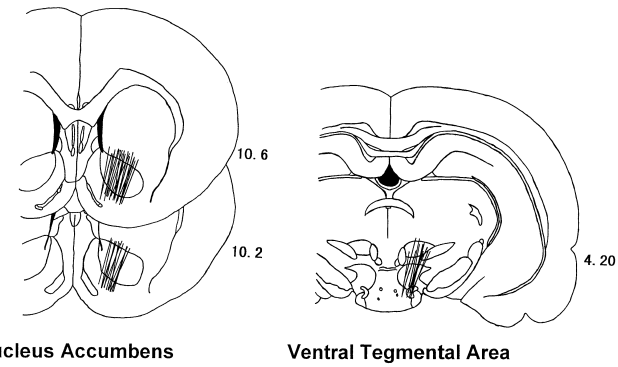

FIG. 1. Locations of the probes in the nucleus accumbens (left part) and in the ventral tegmental area (right part). The planes are modified to a serious of two or three sections for each brain area from the atlas of Paxinos and Watson (21) and the coordinates represent anterior distance (mm) from the interaural line.

were taken every 25 min for quantification of dopamine and GABA. Drugs were injected either intravenously, via the exteriorized jugular cannula, or intraperitoneally (for drug suspensions), the mean of the last three samples before the drug injection being taken as the baseline value. The probes had an in vitro recovery of approximately 12% for both dopamine and GABA, but the reported concentrations were not adjusted for recovery in vivo because these estimations are inaccurate (1,15).

Dopamine was separated on an Eicompak CA-5ODS column (5 μm, 4.6 × 150 mm, EICOM) using phosphate buffer (0.1 M) containing octane-sulfonic acid (3.2 mM), EDTA (1.5 mM), and methanol (20%, pH 6.0) as the mobile phase (flow rate 1.0 ml/min). The compounds were measured by electrochemical detection using a glassy carbon working electrode set at +400 mV against a silver–silver chloride reference electrode, giving a detection limit for dopamine of about 0.5 pg per sample.

GABA was determined by precolumn derivatization (10-μl sample) with *o*-phthaldialdehyde/mercaptoethanol regent followed by separation by reversed-phase high-performance liquid chromatography on an Eicompak MA-5ODS column (5 μm, 2.1 × 150 mm, EICOM) perfused under isocratic condition at the flow rate of 0.18 ml/min. The mobile phase was phosphate buffer (0.05 M) containing methanol (50%, pH 3.5 adjusted by phosphoric acid). A Hitachi (Tokyo, Japan) model 1050 fluorescence spectrophotometer with excitation wavelength set at 340 nm and emission cutoff filter set at 445 nm was used. The limit of detection was about 10 pg per sample.

Drugs

Drugs used were the α_2-adrenoceptor agonist clonidine hydrochloride (Research Biochemicals International), the α_2-adrenoceptor antagonist idazoxan hydrochloride (Research Biochemicals International), the benzodiazepine receptor agonist midazolam (Dormicum, Yamanouchi Pharmaceutical), the $GABA_A$ receptor antagonist (−)-bicuculline methylbromide (Research Biochemicals International), the benzodiazepine receptor antagonist flumazenil (Ro 15-1788, Hoffmann–La Roche), and the benzodiazepine receptor inverse agonist β-carboline-3-carboxylate ethyl ester (β-CCE, Research Biochemicals International). Midazolam, clonidine, idazoxan, bicuculline, and flumazenil were dissolved in 0.9% w/v NaCl solution (saline) for intravenous injections. β-CCE was suspended in a 10% solution of polyethylene glycol in saline and injected intraperitoneally. The doses of the drugs employed in this study were mostly based on our previous studies (18,30).

Histology

At the end of the experiment, each rat was deeply anesthetized with sodium pentobarbitone (80 mg/kg) and perfused transcardially with 10% formal saline. 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 levels, and the method of summary measures to serial measurements (16) was employed for statistical evaluation. In short, the area under the curve was calculated by adding the areas under the graph between each pair of consecutive observations (interval = 25 min), providing the so-called summary measure per rat. The latter values were averaged across all subjects, and the resulting means were statistically analyzed, using one-way analysis of variance (ANOVA) followed by a post hoc Newman–Keuls test or Student's *t*-test, when appropriate. Statistical significance was considered when $p < 0.05$.

RESULTS

Effects of Clonidine and Idazoxan on Dopamine Release

The concentration of dopamine in dialysates of the nucleus accumbens reached a stable baseline value of 8.3 ± 0.6 pg/25 min (mean ± SEM), n = 6) approximately 4 h after probe insertion and was not affected by saline (1 ml IV) over the ensuing 4 h (Fig. 2A).

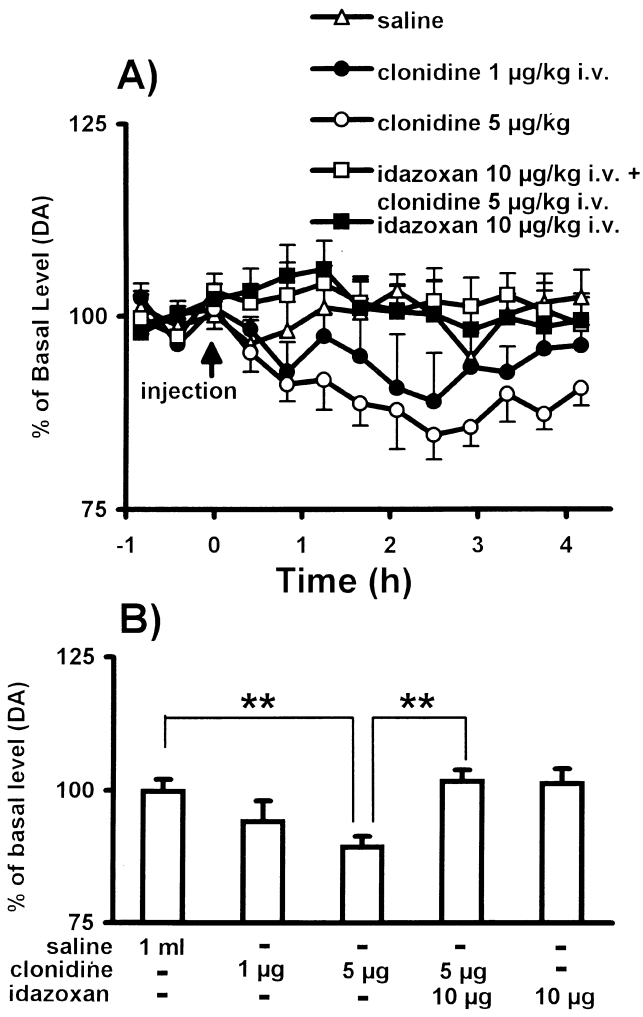

FIG. 2. Effects of intravenous injection of saline, clonidine, and idazoxan on dialysate concentrations of dopamine (DA) in the nucleus accumbens [ANOVA: $F(4, 29) = 7.91$, $p < 0.01$; post hoc values: $^{**}p < 0.01$]. △ = saline; ● = clonidine 1 μg/kg; ○ = clonidine 5 μg/kg; ■ = idazoxan 10 μg/kg; □ = clonidine 5 μg/kg + idazoxan 10 μg/kg. Values (mean ± SEM) represent data from six rats. Time-dependent effects are shown in A, whereas overall effects in a 250-min observation period after injection are shown in B.

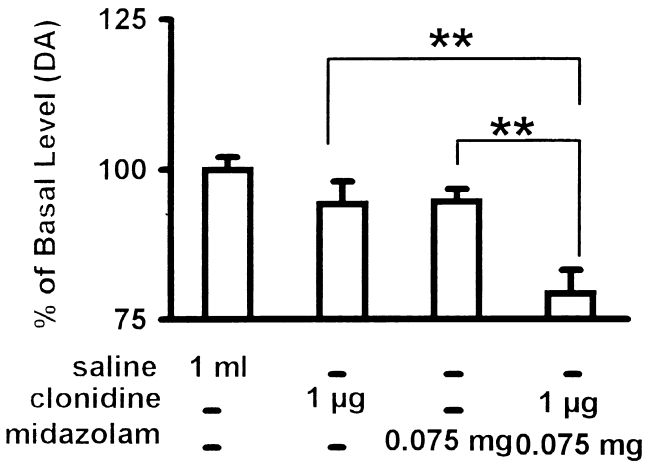

FIG. 3. Effects of intravenous injection of saline, 1 μg/kg clonidine, 0.075 mg/kg midazolam and the combination of 1 μg/kg clonidine and 0.075 mg/kg midazolam on the concentrations of dopamine (DA) in dialysates of the nucleus accumbens [ANOVA: $F(3, 23) = 15.94$, $p < 0.01$; post hoc values: $^{**}p < 0.01$]. The data are expressed as the mean of overall change in a 250-min observation period after injection ($n = 6$). Vertical bars indicate SEM.

Clonidine (5 μg/kg IV, $n = 6$) significantly decreased the dopamine concentration in the dialysate of the nucleus accumbens to a maximum of 16% at its peak effect that occurred about 2.5 h after injection; the average decrease was 11% throughout the experiment ($p < 0.01$ vs. saline, Newman–Keuls test); a smaller dose, 1 μg/kg IV ($n = 6$), produced no change. The effect of clonidine (5 μg/kg IV) was prevented ($p < 0.01$, Newman–Keuls test) by a dose of idazoxan (10 μg/kg IV, $n = 6$) that itself did not affect the concentration of dopamine (Fig. 2A and B).

Effects of Clonidine and Midazolam Combination on Dopamine Release

When an ineffective dose of clonidine (1 μg/kg IV) was combined with an ineffective dose of midazolam (0.075 mg/kg IV, $n = 6$), a benzodiazepine receptor agonist, a 21% decrease in dopamine release of the nucleus accumbens was seen throughout the whole observation period ($p < 0.01$, Newman–Keuls test, Fig. 3).

Effects of Bicuculline, β-CCE and Flumazenil on Clonidine-Induced Dopamine Reduction

Clonidine (5 μg/kg IV)-induced decrease in dopamine release of the nucleus accumbens was abolished ($p < 0.01$, Newman–Keuls test) by a dose of bicuculline (1 mg/kg IV, $n = 6$),

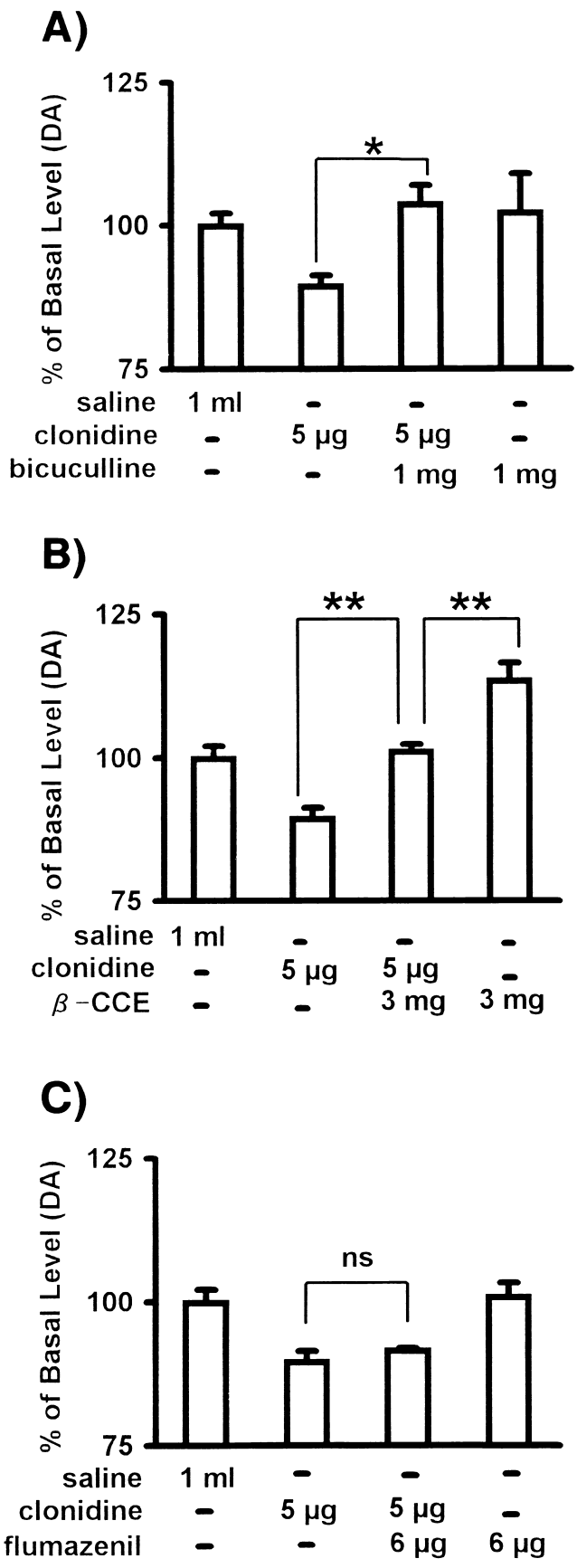

FIG. 4. Effects on clonidine (5 μg/kg IV)-induced reduction in dopamine (DA) release in the nucleus accumbens of (A) 1 mg/kg IV bicuculline [ANOVA: $F(3, 25) = 3.05$, $p < 0.05$; post hoc value: $^{*}p < 0.05$], (B) 3 mg/kg IP β-carboline-3-carboxylate ethyl ester [β-CCE, ANOVA: $F(3, 23) = 36.30$, $p < 0.01$; post hoc values: $^{**}p < 0.01$], and (C) 6 μg/kg IV flumazenil [ANOVA: $F(3, 23) = 8.49$, $p < 0.01$; post hoc test revealed no significant difference between clonidine with and without flumazenil]. The data are expressed as the mean of overall change in a 250-min observation period after injection ($n = 6$–8). Vertical bars indicate SEM.

a $GABA_A$ receptor antagonist, that itself did not affect the concentration of dopamine in the dialysates ($n = 8$, Fig. 4A); a higher dose (10 mg/kg IV) of bicuculline alone produced a nonsignificant small (10%) increase in the dialysate concentration of dopamine (110.2 ± 14.9%, $n = 6$).

β-CCE (3 mg/kg IP, $n = 6$), a benzodiazepine receptor inverse agonist, increased dopamine release, and when coadministered with clonidine (5 μg/kg IV, $n = 6$), the effects of clonidine were overruled ($p < 0.01$, Newman–Keuls test; Fig. 4B).

The clonidine-induced decrease in dopamine release was not significantly affected by a dose of flumazenil (6 μg/kg IV, $n = 6$), a benzodiazepine receptor antagonist, that itself failed to affect the concentration of dopamine in the dialysates ($n = 6$, Fig. 4C).

Effects of Clonidine on GABA Release

The concentrations of GABA in the dialysate of the nucleus accumbens and the ventral tegmental area reached a stable baseline value of 21.7 ± 9.0 pg/25 min ($n = 8$) and of 31.5 ± 8.8 pg/25 min ($n = 6$), respectively, approximately 24 h after probe insertion and were not affected by saline (1 ml IV) over the ensuing 4 h, although there was a small decline in the baseline value of GABA in the ventral tegmental area (Fig. 5).

Clonidine (5 μg/kg IV, $n = 8$) significantly increased the dialysate concentration of GABA in the nucleus accumbens when compared with saline control (*t*-test: $t = 1.95$; $p < 0.05$). The peak effect of clonidine occurred approximately 2 h after injection and remained effective at least over the observation period (Fig. 5, upper part). Conversely, clonidine failed to influence the dialysate concentration of GABA in the ventral tegmental area ($n = 6$, Fig. 5, lower part).

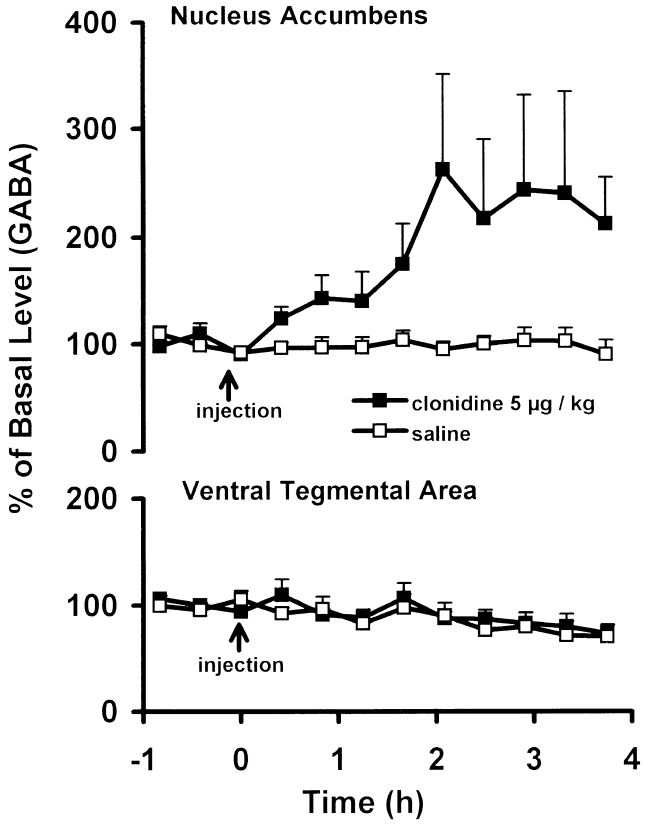

FIG. 5. Effects of intravenous injection of saline and clonidine on the dialysate concentrations of γ-aminobutyric acid (GABA) of the nucleus accumbens (upper part) and of the ventral tegmental area (lower part). ■ = saline; □ = clonidine 5 μg/kg. Values (mean ± SEM) represent data from six to eight rats.

Miscellaneous

None of the treatments produced any behavioral change, apart from the fact that all subjects were more or less sedated throughout the whole observation of 250 min across the various experiments.

DISCUSSION

The present study shows that intravenous administration of the α_2-adrenoceptor agonist clonidine produced a 16% decrease in dopamine release in the nucleus accumbens which peaked about 2.5 h after injection. This slow peak effect of clonidine on dopamine may suggest that the induced effect is mediated by indirect mechanisms. This effect appears to be mediated by α_2-adrenoceptors because it was blocked by the selective α_2-adrenoceptor antagonist idazoxan. However, a possible involvement of imidazoline receptors cannot be excluded, because both clonidine and idazoxan are known to act at these receptors as well (2,4). Although the involved adrenoceptors might be located presynaptically on dopaminergic terminals, a situation that is known to occur in the striatum (31), this is unlikely in view of the finding that bicuculline abolished the effects of clonidine upon dopamine.

We have previously reported that 0.15 mg/kg midazolam (IV) decreases dopamine release from the rat nucleus accumbens (18). In the present study, a small, ineffective dose of clonidine (1 μg/kg IV) significantly decreased the release of dopamine in the nucleus accumbens, when combined with an ineffective dose of midazolam (0.075 mg/kg IV), suggesting the presence of an interaction between the effects of clonidine and midazolam; however, the available data are insufficient to state that this interaction is additive or synergistic.

As mentioned in the introductory paragraphs, there is evidence that the ability of midazolam to reduce the release of mesolimbic dopamine is mediated via the $GABA_A$/benzodiazepine complex, especially via the benzodiazepine binding site (18). The present study shows that the $GABA_A$/benzodiazepine complex, especially the $GABA_A$ binding site appears to form part of the sequence of events that is triggered by clonidine in an α_2-adrenergic-specific manner and that ultimately results in a decreased release of dopamine in the nucleus accumbens. First, the effects of clonidine on dopamine release were antagonized by the $GABA_A$ receptor antagonist bicuculline in a dose (1 mg/kg IV) that itself does not affect dopamine release in the nucleus accumbens, implying that clonidine might have produced an increased GABAergic activity (see also: below). Second, β-CCE, an inverse agonist of benzodiazepine receptors, counteracted the effects of clonidine in a dose of β-CCE (3 mg/kg IP) that per se enhances dopamine release in the nucleus accumbens (18); this effect can be ascribed to the ability of inverse agonists of benzodiazepine receptors to decrease chloride conductance and, accordingly, to counteract effects of an increased GABAergic activity (28). Third, flumazenil, an antagonist of benzodiazepine receptors, failed to affect the effects of clonidine in a dose of flumazenil (6 μg/kg IV) that effectively antagonizes both midazolam-induced decrease and β-CCE–induced increase in dopamine release, but does not affect the release of dopamine itself (18,30); this effect can be ascribed to the inability of benzodiazepine receptor antagonists to affect the $GABA_A$/benzodiazepine complex when given alone (28).

Because the above-mentioned findings suggested that the effects of clonidine upon the release of mesolimbic dopamine were mediated via an increased activity at $GABA_A$ binding sites, attention was focused on two putative sites of action, namely the nucleus accumbens and the ventral tegmental area. The present study reveals that clonidine significantly enhanced the release of GABA in the nucleus accumbens, but not in the ventral tegmental area. Although there is no direct proof that GABA was solely released from neurons, it is likely that the increase in the nucleus accumbens relates to increased neurotransmission, given the fact that there are at least four possible, neuronal target sites. First, clonidine might have activated α_2-adrenoceptors that are located presynaptically on GABAergic terminals in the nucleus accumbens and, accordingly, enhanced the release of GABA, being a situation that is known to occur in the hippocampus and the cortex (17,23,24). Second, clonidine might have activated α_2-adrenoceptors that are located on GABAergic cell bodies in the nucleus accumbens; for it is known that this nucleus contains numerous intrinsic GABAergic neurons. Third, clonidine might have activated α_2-adrenoceptors that are located on cell bodies of GABAergic neurons that arise in the ventral pallidum and terminate in the nucleus accumbens (3). Finally, clonidine might have activated α_2-adrenoceptors that are located on cell bodies of adrenergic neurons that directly or indirectly innervate GABAergic neurons in the nucleus accumbens.

Despite the fact that none of the above-mentioned possibilities can be excluded, the present study suggests that the α_2-adrenoceptor agonist clonidine directly or indirectly enhances the release of GABA in the nucleus accumbens that, in turn, may decrease the release of mesolimbic dopamine. Because a hypofunction of mesolimbic dopamine results in sedation (22), the present study opens the perspective that clonidine's ability to decrease the release of mesolimbic dopamine via enhancing the release of mesolimbic GABA underlies its capacity to enhance sedation induced by benzodiazepines. In this context, it is interesting to note that an increase in the release of striatal dopamine has been found to enhance the effects of anesthetics (27). These data, together with the present findings, underline the well-known fact that the dopaminergic activity in the dorsal striatum mediates functions that are different from those mediated by the dopaminergic activity in the ventral striatum including the nucleus accumbens (5,14).

ACKNOWLEDGEMENTS

This study was supported in part by Research Grants on Health Sciences from the Ministry of Health and Welfare and from the Ministry of Education, Science and Culture (Japan). We thank Hoffmann–La Roche and Yamanouchi for the gift of their compounds.

REFERENCES

1. Benveniste, H.; Hansen, A. J.; Ottosen, N. S.: Determination of brain interstitial concentration by microdialysis. J. Neurochem. 52:1741–1750; 1989.
2. Bricca, G.; Zhang, J.; Greney, H.; Dontenwill, M.; Stutuman, J.; Belcourt, A.; Bousquet, P.: Relevance of the use of [^{3}H]-clonidine to identify imidazoline receptors in the rabbit brainstem. Br. J. Pharmacol. 110:1537–1543; 1993.
3. Churchill, L.; Kalivas, P. W.: A topographically organized gamma-aminobutyric acid projection from the ventral pallidum to the nucleus accumbens in the rat. J. Comp. Neurol. 345:579–595; 1994.
4. Codd, E. E.; Press, J. B.; Raffa, R. B.: Alpha$_2$-adrenoceptors vs. imidazoline receptors: Implications for α_2-mediated analgesia and other non-cardiovascular therapeutic uses. Life Sci. 56:63–74; 1994.
5. Cools, A. R.; Berger, H. J. C.; Buytenhuijs, E. L.; Horstink, M. W. I. M.; Van Spaendonck, K. P. M.: Manifestations of switching disorders in animals and men with dopamine deficits in A_{10} and/or A_9 circuitries. In: Wolters, E. L.; Scheltens, P., eds. Mental disfunction in Parkinson's disease. Amsterdam: Vrije Universiteit; 1993:49–67.
6. Cools, A. R.; van den Bos, R.; Ploeger, G.; Ellenbroek, B. A.: Gating function of noradrenaline in the ventral striatum: Its role in behavioural responses to environmental and pharmacological challenges. In: Willner, P.; Scheel-Krüger, J., eds. The mesolimbic dopamine system: From motivation to action. Chichester: John Wiley & Sons; 1991:141–173.
7. Di Chiara, G.; Imperato, A.: Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA. 85:5274–5278; 1988.
8. Finlay, J. M.; Damsma, G.; Fibger, H. C.: Benzodiazepine-induced decreases in extracellular concentrations of dopamine in the nucleus accumbens after acute and repeated administration. Psychopharmacology (Berlin) 106:202–208; 1992.
9. Horváth, G.; Szikszay, M.; Rubicsek, G.; Benedek, G.: An isobolographic analysis of the hypnotic effects of combinations of dexmedetomidine with fentanyl or diazepam in rats. Life Sci. 50:215–220; 1992.
10. Invernizzi, R.; Pozzi, L.; Samanin, R.: Release of dopamine is reduced by diazepam more in the nucleus accumbens than in the caudate nucleus of conscious rats. Neuropharmacology 30:575–578; 1991.
11. Johnston, R. R.; Way, W. L.; Miller, R. D.: Alteration of anesthetic requirement by amphetamine. Anesthesiology 36:357–363; 1972.
12. Johnston, R. R.; White, P. F.; Way, W. L.; Miller, R. D.: The effect of levodopa on halothane anesthetic requirements. Anesth. Analg. 54:178–181; 1975.
13. Kanto, J.: Benzodiazepines as oral premedicants. Br. J. Anaesth. 53:1179–1188; 1981.
14. Koshikawa, N.: Role of the nucleus accumbens and the striatum in the production of turning behaviour in intact rats. Rev. Neurosci. 5:331–346; 1994.
15. Lindefors, N.; Amberg, G.; Ungerstedt, U.: Intracerebral microdialysis, I. Experimental studies of diffusion kinetics. J. Pharmacol. Methods. 22:141–156; 1989.
16. Matthews, J. N. S.; Altman, D. G.; Campbell, M. J.; Royston, P.: Analysis of serial measurements in medical research. Br. Med. J. 300:230–235; 1990.
17. Maura, G.; Pittaluga, A.; Ulivi, M.; Raiteri, M.: Enhancement of endogenous GABA release from rat synaptosomal preparation is mediated by α_2-autoreceptors. Eur. J. Pharmacol. 157:23–29; 1988.
18. Murai, T.; Koshikawa, N.; Kanayama, T.; Takada, K.; Tomiyama, K.; Kobayashi, M.: Opposite effects of midazolam and β-carboline-3-carboxylate ethyl ester on the release of dopamine from rat nucleus accumbens measured by in vivo microdialysis. Eur. J. Pharmacol. 261:65–71; 1994.
19. Murai, T.; Kyoda, N.; Misaki, T.; Takada, K.; Sawada, S.; Machida T.: Effects of clonidine on intravenous sedation with midazolam. Anesth. Prog. 42:135–138; 1995.
20. Nurse, B.; Russell, V. A.; Taljaard, J. J. F.: Effects of chronic desipramine treatment on adrenoceptor modulation of [^{3}H]dopamine release from rat nucleus accumbens slices. Brain Res. 334: 235–242; 1985.
21. Paxinos, G.; Watson, C.: The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.

22. Pijnenburg, A. J. J.; Honig, W. M. M.; van der Heyden, J. A. M.; van Rossum, J. M.: Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. Eur. J. Pharmacol. 35:45–58; 1976.
23. Pittaluga, A.; Raiteri, M.: GABAergic nerve terminals in rat hippocampus possess α_2-adrenoceptors regulating GABA release. Neurosci. Lett. 76:363–367; 1987.
24. Pittaluga, A.; Raiteri, M.: Clonidine enhances the release of endogenous γ-aminobutyric acid through alpha-2 and alpha-1 presynaptic adrenoceptors differentially located in rat cerebral cortex subregions. J. Pharmacol. Exp. Ther. 245:682–686; 1988.
25. Salonen, M.; Reid, K.; Maze, M.: Synergistic interaction between α_2-adrenergic agonists and benzodiazepines in rats. Anesthesiology 76:1004–1011; 1992.
26. Segal, I. S.; Jarvis, D. J.; Duncan, S. R.; White, P. F.; Maze, M.: Clinical efficacy of oral-transdermal clonidine combinations during the perioperative period. Anesthesiology 74:220–225; 1991.
27. Segal, I. S.; Walton, J. K.; Irwin, I.; Delanney, L. E.; Ricaurte, G. A.; Langston, J. W.; Maze, M.: Modulating role of dopamine on anesthetic requirements. Eur. J. Pharmacol. 186:9–15; 1990.
28. Sieghart, W.: $GABA_A$ receptors: Ligand-gated Cl^- ion channel modulated by multiple drug-binding sites. Trends Pharmacol. Sci. 13:446–450; 1992.
29. Stoelting, R. K.; Celasser, C. W.; Marty, R. C.: Effect of cocaine administration on halothane MAC in dogs. Anesth. Analg. 54:422–424; 1975.
30. Takada, K.; Murai, T.; Kanayama, T.; Koshikawa, N.: Effects of midazolam and flunitrazepam on the release of dopamine from rat striatum measured by *in vivo* microdialysis. Br. J. Anaesth. 70:181–185; 1993.
31. Trendelenburg, A.-U.; Starke, K.; Limberger, N.: Presynaptic α_{2A}-adrenoceptors inhibit the release of endogenous dopamine in rabbit caudate nucleus slices. Naunyn Schmiedebergs Arch. Pharmacol. 350:473–481; 1994.
32. Unnerstal, J. R.; Kopatijic, T. A.; Kuhar, M. J.: Distribution of alpha$_2$-agonist binding sites in the rat and human central nervous system; Analysis of some functional correlates of the pharmacological effects of clonidine and related adrenergic agents. Brain Res. Rev. 319:69–101; 1984.
33. Zetterström, T.; Fillenz, M.: Local administration of flurazepam has different effects on dopamine release in striatum and nucleus accumbens: A microdialysis study. Neuropharmacology 29:129–134; 1990.