Neuropsychopharmacology (2003) 28, 1198–1206

www.neuropsychopharmacology.org

Heat Loss, Sleepiness, and Impaired Performance after Diazepam Administration in Humans

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In spite of the accumulation of knowledge regarding the neuropharmacological action of benzodiazepines (Bz), the physiological process by which their sedative/hypnotic effects are induced remains poorly understood. We conducted a single-blind, crossover trial to evaluate the role of the thermoregulatory process in sleepiness and impaired psychomotor performance induced by a standard Bz, diazepam (DZP). Each of the eight healthy young male volunteers (mean age, 19.75 years; range, 18–23 years) was given a single oral dose of either 5 or 10 mg of DZP or placebo 12 h after his average sleep onset time. Changes in plasma DZP concentration, proximal body temperature (p-BT), distal body temperature (d-BT), subjective sleepiness measured by the Visual Analog Scale and Stanford Sleepiness Scale, and psychomotor performance measured by Choice Reaction Time were monitored under a modified constant routine condition in which various factors affecting thermoregulation, alertness, and psychomotor performances were strictly controlled. Orally administered DZP induced a significant transient decrease in p-BT and psychomotor performance as well as an increase in d-BT and subjective sleepiness. Distal–p-BT gradient (DPG; difference between d-BT and p-BT), which is an indicator of blood flow in distal skin regions, showed a strong positive correlation with the plasma DZP concentration, indicating that DZP in clinical doses promotes heat loss in a dose-dependent manner. The DPG also correlated positively with the magnitude of subjective sleepiness and impaired psychomotor performance. These findings indicate that the sedative/hypnotic effects of Bz could be due, at least in part, to changes in thermoregulation, especially in the process of heat loss, in humans.
Neuropsychopharmacology (2003) **28,** 1198–1206. advance online publication, 2 April 2003; doi:10.1038/sj.npp.1300160

Keywords: benzodiazepine; diazepam; thermoregulation; GABA; sleepiness; psychomotor performance

INTRODUCTION

Benzodiazepine (Bz) is known to have sedative/hypnotic effects on the brain function represented by the induction of sleepiness and decline in psychomotor performance. Bz-binding site ligands cause allosteric modulation of $GABA_A$–Cl^- channel complex, which subsequently induces an opening of ion channel pore and Cl^- flows across the neuronal membrane (Barnard *et al*, 1998; McKernan and Whiting, 1996; Polc, 1988). The principal characteristic of BZP action has been assumed to be the inhibition of other neuronal systems, including the monoaminergic and/or glutaminergic projections, which could account in part for its sedative effects and side effects related to attention and balance, for example. However, the 'physiological process' underlying Bz's sedative/hypnotic effects remains poorly understood.

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Received 29 August 2002; revised 12 December 2002; accepted 18 December 2002
Online publication: 06 January 2003 at http://www.acnp.org/citations/Npp010603449

Growing evidence suggests that thermoregulation, especially the process of heat loss, is a key pathway generating sleepiness and impaired psychomotor performance in humans. The circadian profiles of both alertness and psychomotor performance are mirror images of the endogenous core body temperature (core BT) rhythm, with a trough of alertness and psychomotor performance levels occurring during the several hours around the minimum core BT (Daurat *et al*, 1993; Drummond *et al*, 2001; Gillberg *et al*, 1994; Leproult *et al*, 1997; Monk *et al*, 1997). Recent studies have shown that the sleep-producing mechanism in humans is preceded by increased heat loss (Campbell and Broughton, 1994; Gilbert *et al*, 1999; Krauchi *et al*, 1999, 1997) and that various sleep-producing manipulations act, at least in part, by enhancing the heat loss mechanism (Deacon *et al*, 1994; Dorsey *et al*, 1999; Gilbert *et al*, 1999; Krauchi *et al*, 1999). Furthermore, physiological sleepiness has been shown to cause a decrease in psychomotor performance (De Gennaro *et al*, 2001; De Valck and Cluydts, 2001; Hood and Bruck, 1996; Kinnari *et al*, 2000; Nakano *et al*, 2000; Nave *et al*, 2002; Porcu *et al*, 1998; Schulz *et al*, 1997; Thomas *et al*, 2000; Wesensten *et al*, 2002).

We report here that a standard Bz, diazepam (DZP), induced a sedative effect in humans via the thermoregulatory system such that the DZP-related sleepiness and

impaired psychomotor performance progressed in parallel with the magnitude of heat loss. The goal of our study was to evaluate the effects of DZP administered at clinical doses on thermoregulation, sleepiness, and psychomotor performance using high time resolution in young healthy volunteers under a modified constant routine condition (Mills *et al*, 1978), in which various masking effects produced by physical movement, posture, calorie intake, clothing, ambient temperature, and environmental light intensity (Campbell and Dawson, 1990; Rietveld *et al*, 1993) are strictly controlled.

METHODS AND SUBJECTS

Subjects

Before enrollment in the present study, healthy volunteers under 25 years of age were screened by three physicians who conducted rigorous physical and psychological evaluations of each subject. Volunteers were surveyed to exclude the following: irregularity in the sleep–wake pattern according to a self-registering sleep diary kept for 2 weeks, a history of physical disease that could affect sleep states suggested by the International Classification of Sleep Disorders (ICSD, 1990), for example, chronic headaches, asthma, gastroesophageal reflux, peptic ulcer disease, etc, history of psychiatric disease identified by the Mini-International Neuropsychiatric Interview (MINI) (Sheehan *et al*, 1998) or a structured diagnostic psychiatric interview for DSM-IV disorders (including depression and anxiety disorders), use of medications that might modify sleep states during the prior 3 months, any airline travel that could cause jet lag during the prior 6 months, and any abnormal hematology or urinalysis findings. Eight healthy male volunteers with a mean age of 19.75 years (range 18–23) who gave written informed consent participated in the study.

Experimental Procedures

General setting. The study protocol is illustrated in Figure 1. All subjects abstained from drugs, tobacco, alcohol, heavy exercise, and medications for at least 7 days before the study (prestudy period). During this prestudy period, all subjects were asked to keep their daily routine at home but to make sure the light intensity during the time in bed was kept under 10 lux. Sleep quality and regularity were assessed by means of an actigraph (AMI Inc., Ardsley, NY) fitted to the nondominant wrist of each subject; the data were analyzed for computer-calculated sleep–wake determinations by Cole's algorithm (Cole *et al*, 1992). For each subject, the sleep onset times calculated by actigraph during the prestudy period were averaged, and the average onset time was defined as 0000 h. Each subject participated in three experiments (two drug experiments and one placebo experiment) that were conducted at 2-week intervals. On the day before each experiment, the subject entered the sleep laboratory at 8 h before 0000 h (−0800 h). In the period before −0600 h, the subject donned cotton pajamas provided by the laboratory to control for the influence of clothing on thermoregulation. At −0600 h, a 750-kcal meal and as much water as desired was given to each subject. From the time after the meal and for over 22 h until the end of the study at 1630 h the next day, subjects rested in the supine position on a reclining seat during the wake time. Standing and walking were prohibited, and movement of the limbs was discouraged. An adjoining room was fitted with a portable toilet, movement to the toilet was assisted, and sitting was permitted while the toilet was being used. Subjects were allowed to recline and sleep on a bed in the laboratory only at 0000–0800 h. During the sleep time, each subject underwent polysomnography and was confirmed to have no sleep disorder as defined by the ICSD (1990). Sleeping was forbidden outside the sleep period. Participants were subjected to continuous EEG monitoring, and two or more laboratory workers were always present to observe the subjects and provide assistance. Laboratory lighting was maintained at less than 10 lux during the sleep period and at 100 lux near the subjects' eyes at other times to avoid the alerting and alleviating effects of bright light on sleepiness and psychological performance (Campbell and Dawson, 1990). The room temperature was maintained at $23 \pm 1^{\circ}C$ throughout the study period.

Drug administration. On the day of the experiment, the subject ingested a 100-kcal meal and 100 ml of orange juice at 0830 h to ensure alimentary canal absorption of DZP and to suppress the influence of calorie intake on temperature and psychomotor performance. The experiments tested the

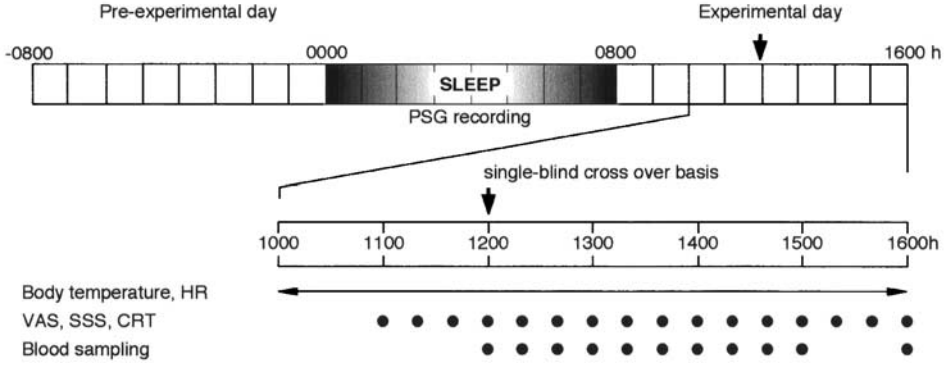

Figure 1 Experimental protocol. PSG: Polysomnography, VAS: Visual Analog Scale, SSS: Stanford Sleepiness Scale, CRT: Choice Reaction Time. Vertical arrows indicate the time of either DZP or placebo administration, which occurred 12 h after each subject's average sleep onset time (1200 h).

subject's responses to placebo (lactose), 5 mg of DZP, or 10 mg of DZP, all of which were packed in indistinguishable gelatin capsules and were administered with 100 ml of water at 1200 h. The study was conducted in a randomized, single-blind, crossover fashion.

Evaluation of plasma concentration of DZP and desmethyldiazepam. Blood samples were used to monitor plasma DZP and its active metabolite desmethyldiazepam (dmDZP). Samples were drawn every 20 min from 1200 to 1500 h and again at 1600 h via intravenous catheter placed in a forearm vein at 0900 h. Blood samples were immediately centrifuged at 3000 rpm for 5 min, and the plasma was collected and frozen at −20°C for later assay. Plasma concentrations of DZP and dmDZP were determined according to a high-performance liquid chromatography (HPLC) method previously described (Miura *et al*, 2002; Nagasaki *et al*, 1999) but with some modifications. The apparatus used for HPLC was the EP-300 chromatography pump (EICOM, Kyoto, Japan) equipped with an EICOM NOD-10 NOx detector. The wavelength was set at 230 nm. Test samples were introduced with a 234 autoinjector (Gilson Inc., Wisconsin) at an effective volume of 75 μl. The HPLC column used was a Grand pack ODS-5 NK stationary phase (5 μm, Masis Inc. Owani, Japan). The column temperature was maintained at 25.0°C in an EICOM ATC-300 column oven. The mobile phase consisted of 0.5% KH_2PO_4 (pH 4.5)–acetonitrile (60 : 40, v/v), which was degassed in an EICOM DG-300 degasser prior to use. The flow rate was 1.0 ml/min. All solvents used were of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). After flunitrazepam (320 ng) in methanol (10 μl) was added to plasma samples (1 ml) as an internal standard, the plasma samples were diluted with 5 ml water, and the solution was mixed briefly. The mixture was applied to a Sep-Pak CN cartridge (Waters, Bedford, MA) that had been activated previously with 10 ml acetonitrile and water. The cartridge was then washed with 10 ml water and 5 ml 20% acetonitrile in water. The desired fraction was eluted with 5 ml 70% acetonitrile in water. The elute was evaporated in a vacuum at 40°C by rotary evaporator (Tokyo Rikakikai, Tokyo, Japan). The residue was dissolved in 50 μl methanol, and 100 ml mobile phase was added. The samples were injected into the HPLC apparatus. The ratios of drug to internal standard were calculated from the recorded peaks. The results obtained from spiked plasma samples containing known amounts of drug were calculated on the basis of linear regression analysis.

Evaluation of heat loss after DZP administration. In the period prior to −0600 h, skin-temperature thermistors were attached to the left and right wrist area and the instep of both feet. A rectal thermistor (polyethylene-covered thermoprobe, accuracy within 0.01°C) was inserted 10 cm into the subject's rectum. The thermistors were connected to an ambulatory temperature monitoring system (Kohden Medical Inc., Tokyo, Japan), and sampling occurred at 1-min intervals. All temperature recordings taken on the day of the experiment were later collapsed into 20-min bins from 1100 to 1600 h. The data at 1200 h were averaged for 10 min just before drug administration to control for the acute drug effects. After that, bin data from each subject were expressed relative to the data at 1200 h. Proximal body temperature (p-BT), that is, rectal temperature, and distal body temperature (d-BT), that is, average skin temperature from four sites were used to calculate distal−proximal BT gradient (DPG, difference between d-BT and p-BT) as an indicator of blood flow in distal skin regions (Krauchi *et al*, 1999; Rubinstein and Sessler, 1990). For further analysis, the change in DPG (ΔDPG) induced by DZP was defined as the difference in the corresponding values between the DZP and placebo experiments at each point of measure.

Evaluation of subjective sleepiness and psychomotor performance. Subjective sleepiness and psychomotor performance were monitored every 20 min from 1000 to 1600 h. During the first 5 min of each 20 min epoch, the Visual Analog Scale (VAS) (Folstein and Luria, 1973), Stanford Sleepiness Scale (SSS) (Hoddes *et al*, 1973), and Choice Reaction Time (CRT) (Sekine *et al*, 2001) were applied consecutively.

The VAS technique, self-registering on a line 100 mm in length ranging from 'very alert' to 'very sleepy,' and the SSS technique, self-registering by a seven-grade scale ranging from 'feeling active, vital, alert, wide awake' to 'almost in reverie, cannot stay awake, sleep onset appears imminent,' have been used widely to evaluate the subjective sleepiness.

CRT was used to assess objective psychomotor performance, as reported previously (Sekine *et al*, 2001). Two different brief tones (2000 and 1000 Hz), each with a duration of 100 ms, were generated by an automatic stimulator (Nihon Kohden SS-1449, Nihon Kohden, Tokyo, Japan) and presented at 60 dB (normal hearing level). The 1000 Hz tone was presented 80% of the time and the target 2000 Hz was presented 20% of the time. The order of presentation was randomized. The interstimulus interval was also randomized within the range of 1.0–3.0 s (mean 2.0 s). The subjects were instructed to respond only to the target stimuli (2000 Hz tone) by pressing a button attached to the palm of their right hand with their thumb ('oddball' paradigm). The button-press response was converted to an electrical signal that was monitored and recorded on a personal computer. Reaction time latencies from the target stimuli to button press were averaged for a total of 33 times as the CRT data at each point of measure. VAS and CRT were performed at 1200 h for 5 min just before drug administration. All other VAS and CRT data were expressed relative to the data obtained at 1200 h. For further analysis, the change in either VAS (ΔVAS), SSS (ΔSSS), or CRT (ΔCRT) induced by DZP was defined as the difference in the corresponding values between the DZP and placebo experiments at each point of measure.

Evaluation of heart rate. Heart rate (HR) was monitored continuously from 1000 to 1630 h on the day of the experiment by means of an ambulatory HR monitoring system (AC-301 Activetracer, GMS Corp., Tokyo, Japan), and values were later collapsed into 20-min bins from 1100 to 1600 h. The data at 1200 h were averaged for 10 min just before drug administration. Bin data were expressed relative to the data at 1200 h. For further analysis, the change in HR (ΔHR) induced by DZP was defined as the difference in the corresponding values between the DZP and placebo experiments at each point of measure.

Data Analysis

One- and two-way analyses of variance (factors: time and dose) followed by the Bonferroni's *post hoc* analysis were used to examine differences in BT, VAS, SSS, CRT, and HR values obtained for the placebo and for each dose of DZP, appropriately. Relations between DPG and plasma DZP and dmDZP concentrations, subjective sleepiness, and psychomotor performance were determined by Pearson's correlation analysis. Results are shown as mean $\pm$ SEM. $p<0.05$ was considered statistically significant.

RESULTS

Proximal Body Temperature

Changes in the p-BT for the placebo, 5- and 10-mg DZP experiments are shown in Figure 2a. In comparison with p-BT with administration of placebo, p-BT with administration of DZP at 10 mg was significantly decreased ($F=4.14$, $df=15$, $p<0.0001$) such that the maximum p-BT suppression occurred 80 min after DZP administration for $0.17 \pm 0.04^{\circ}C$ followed by a gradual recovery to the placebo level, which occurred 240 min after DZP administration. A two-phase thermoregulatory effect, that is, short-term hypothermia followed by a hyperthermic phase of 180 min, was observed after administration of DZP at 5 mg; however, this change did not reach a statistically significant level compared to that with administration of placebo ($F=0.90$, $df=15$, $p=0.568$).

Distal Body Temperature

Changes in d-BT for the placebo, 5- and 10-mg DZP experiments are shown in Figure 2b. In comparison with d-BT with administration of placebo, d-BT with administration of DZP at 10 mg was significantly increased ($F=2.42$, $df=15$, $p<0.01$) such that the maximum d-BT elevation occurred 40 min after administration for $1.07 \pm 0.29^{\circ}C$ followed by a gradual recovery to the placebo level, which occurred 120 min after DZP administration. No statistically significant change in d-BT was observed with administration of DZP at 5 mg compared to that with administration of placebo ($F=0.29$, $df=15$, $p=0.996$).

Distal–Proximal BT Gradient

Changes in ΔDPG for the 5- and 10-mg DZP experiments are shown in Figure 2c. The DZP administration at 10 mg induced a significant increase in ΔDPG ($F=2.68$, $df=15$, $p<0.002$) such that the maximum ΔDPG elevation occurred 40 min after DZP administration for $1.17 \pm 0.30^{\circ}C$ followed by a gradual recovery to the placebo level, which occurred 180 min after DZP administration. No statistically significant change in ΔDPG was observed with administration of DZP at 5 mg ($F=0.37$, $df=15$, $p=0.983$).

Plasma DZP and dmDZP Concentrations

Changes in the plasma DZP and dmDZP concentrations for the 5- and 10-mg DZP experiments are shown in Figure 2d. Orally administered DZP was absorbed rapidly with mean t_{max} (time of peak concentration) values of 50.0 ± 7.56 and

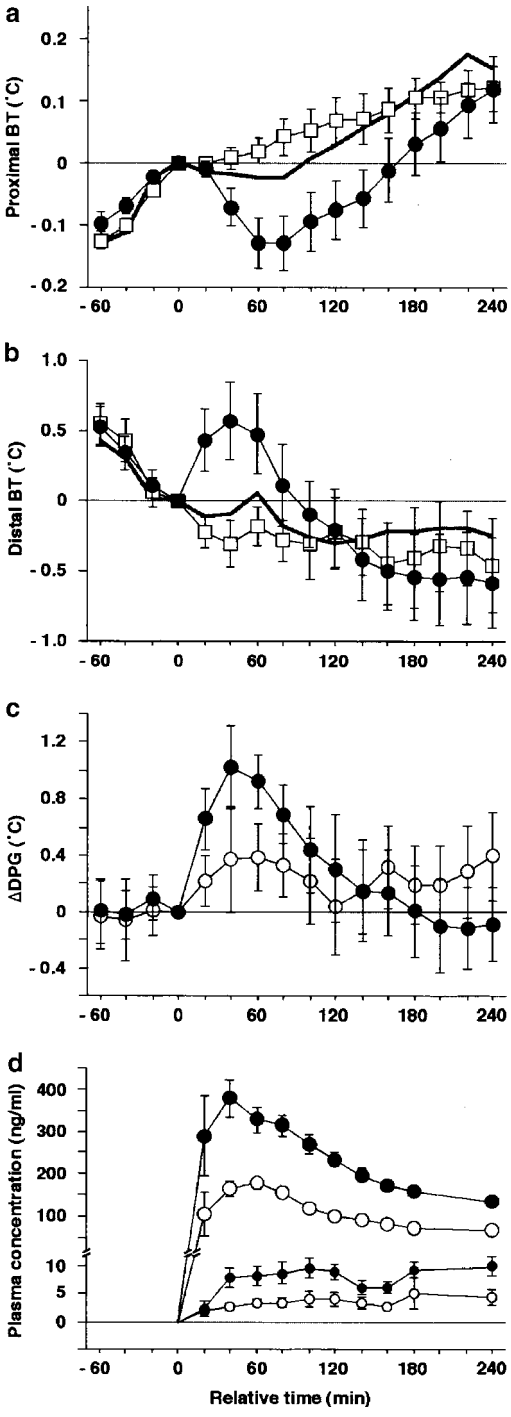

Figure 2 Changes in (a) the proximal BT, (b) distal BT, (c) ΔDPG, and (d) plasma DZP and dmDZP concentrations after oral administration of DZP at 5 and 10 mg. Horizontal bars indicate the time relative to 1200 h of drug administration. Mean $\pm$ SEM values are shown. The proximal and distal BT data are shown as open circles for the 5-mg DZP experiment, closed circles for the 10-mg DZP experiment, and open squares for the placebo experiment. In (a) and (b), SEM values for the 5-mg DZP experiments were omitted for convenience. The ΔDPG in both the 5-mg (open circles) and 10-mg (closed circles) DZP experiments was defined as the difference in the corresponding values between the placebo and DZP experiments at each measured point. In (d), large and small circles correspond to DZP and dmDZP data, respectively. Scales on the *y*-axis were, respectively, modified for DZP and dmDZP data.

45.0 ± 9.06 min as well as mean C_{max} (peak plasma DZP concentration) values of 234.6 ± 23.1 and 484.69 ± 31.11 ng/ml in the 5- and 10-mg DZP experiments, respectively.

Subjective Sleepiness

Changes in ΔVAS for the 5- and 10-mg DZP experiments are shown in Figure 3a. The DZP administration at 10 mg induced a significant increase in ΔVAS ($F = 4.03$, $df = 15$, $p < 0.0001$) and ΔSSS ($F = 4.65$, $df = 15$, $p < 0.0001$), while no statistically significant increases in ΔVAS and ΔSSS were observed with the administration of DZP at 5 mg ($F = 0.87$, $df = 15$, $p = 0.597$ and $F = 1.46$, $df = 15$, $p = 0.133$, respectively). There was a significant intergroup difference in the average VAS score from 20 to 240 min after DZP administration ($F = 9.38$, $df = 2$, $p < 0.01$, Figure 3a) such that the average VAS score in the 10-mg DZP experiment was significantly larger than that in the placebo experiment ($p < 0.001$).

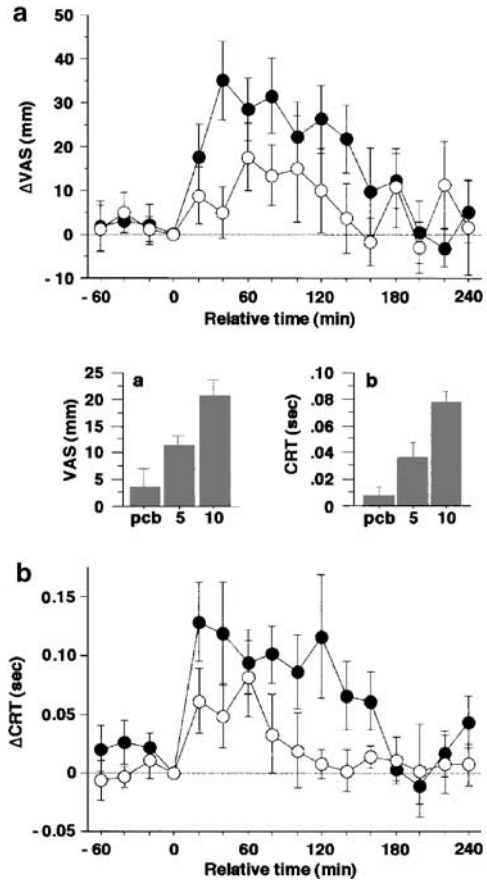

Figure 3 Changes in the ΔVAS and ΔCRT for the 5- and 10-mg DZP experiments. In (a) (upper) and (b) (lower) panels, horizontal bars indicate the time, relative to 1200 h, of drug administration. Mean ± SEM values are shown. The ΔVAS and ΔCRT in both the 5-mg (open circles) and 10-mg (closed circles) DZP experiments were defined as the difference in the corresponding values between the placebo and DZP experiments at each measured point. Middle panels show average values for VAS (a, left) and CRT (b, right) from 20 to 240 min after DZP administration in the placebo (pcb), and 5- and 10-mg DZP experiments.

Psychomotor Performance

Changes in ΔCRT for the 5- and 10-mg DZP experiments are shown in Figure 3b. The DZP administration at 10 mg induced a significant increase in ΔCRT ($F = 3.16$, $df = 15$, $p < 0.001$), while no statistically significant increases in ΔCRT were observed with the administration of DZP at 5 mg ($F = 1.66$, $df = 15$, $p = 0.071$). There was a significant intergroup difference in the average CRT from 20 to 240 min after DZP administration ($F = 14.82$, $df = 2$, $p < 0.0001$, Figure 3b) such that the average CRT in the 10-mg DZP experiments was significantly prolonged compared to that of the placebo experiment ($p < 0.0001$) and the 5-mg DZP experiment ($p < 0.005$).

Heart Rate

Changes in ΔHR for the 5- and 10-mg DZP experiments are shown in Figure 4a. One subject was excluded from the data analysis; his data were lost because of an ECG recording disc error. There was a significant intergroup difference in the average HR from 20 to 240 min after DZP administration ($F = 4.01$, $df = 2$, $p < 0.05$, Figure 4b), such that the average HR in the 10-mg DZP experiment was significantly higher than that of the placebo experiment ($p < 0.02$).

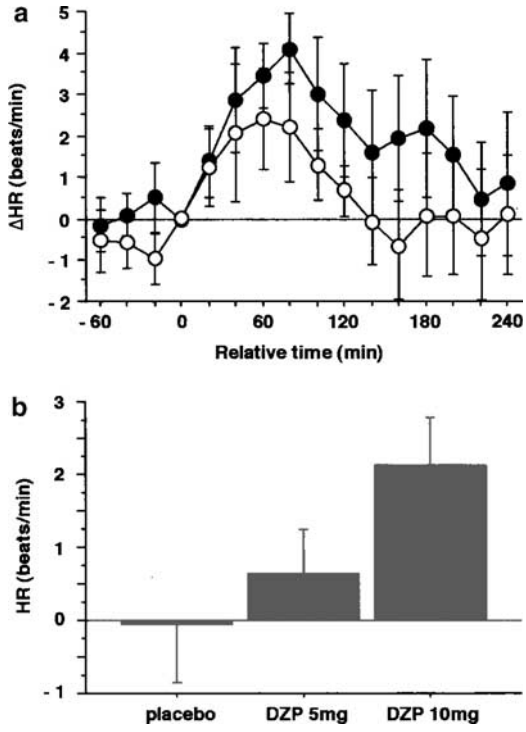

Figure 4 Changes in ΔHR for the 5- and 10-mg DZP experiments. In (a) (upper panel), horizontal bars indicate the time, relative to 1200 h, of drug administration. Mean ± SEM values are shown. ΔHR in both the 5-mg (open circles) and 10-mg (closed circles) DZP experiments were defined as the difference in the corresponding values between the placebo and DZP experiments at each measured point. The lower panel (b) shows average values for HR from 20 to 240 min after DZP administration in the placebo and 5- and 10-mg DZP experiments.

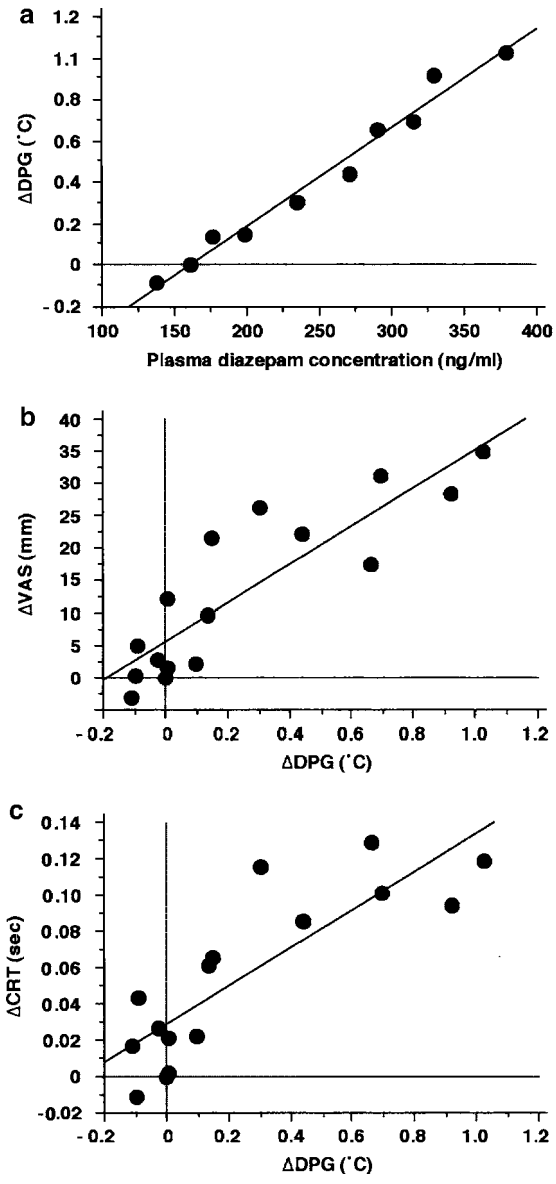

Figure 5 Relation between ΔDPG and (a) plasma DZP concentration, (b) ΔVAS, and (c) ΔCRT in the 10-mg DZP experiment. Pearson's correlation analysis was performed for the mean ΔDPG (*x*-axis) and the mean of plasma DZP concentrations, ΔVAS, or ΔCRT (*y*-axis) at the corresponding time points in the 10-mg DZP experiment.

Relation between DPG, Plasma DZP, and dmDZP Concentrations

There was a strong positive correlation between the mean ΔDPG and the mean plasma DZP concentration at each point of measure in the 10-mg DZP experiment ($r = 0.988$, $p < 0.0001$ (Figure 5a)), whereas correlation was lost in the 5-mg DZP experiment ($r = 0.397$, $p = 0.25$). A positive correlation was also observed between the mean ΔDPG and the mean total plasma concentration for DZP plus dmDZP at each point of measure in the 10-mg DZP experiment ($r = 0.951$, $p < 0.0001$), whereas correlation was also lost in the 5-mg DZP experiment ($r = 0.397$, $p = 0.26$).

Relation between DPG and Subjective Sleepiness

There was a strong positive correlation between the mean ΔDPG and the mean ΔVAS at each point of measure in the 10-mg DZP experiment ($r = 0.876$, $p < 0.0001$) (Figure 5b), whereas the correlation was lost in the 5-mg DZP experiment ($r = 0.331$, $p = 0.21$). There was also a positive correlation between the mean ΔDPG and the mean ΔSSS at each point of measure in the 10-mg DZP experiment ($r = 0.873$, $p < 0.0001$); correlation disappeared in the 5-mg DZP experiment ($r = 0.452$, $p = 0.08$).

Relation between DPG and Psychomotor Performance

There was a strong positive correlation between the mean ΔDPG and the mean ΔCRT at each point of measure in the 10-mg DZP experiment ($r = 0.842$, $p < 0.0001$) (Figure 5c); the correlation was not as strong in the 5-mg DZP experiment ($r = 0.636$, $p < 0.01$).

COMMENT

We showed that DZP administered orally in a clinical dose of 10 mg induced a significant increase in skin temperature and decrease in rectal temperature, resulting in a significant increase in DPG. We know that these thermoregulatory changes were not induced by physical activity, muscle tone, posture changes, or sleep itself because the possible effects of these factors on thermoregulatory parameters were controlled with the use of the modified constant routine protocol (Mills *et al*, 1978). DPG has been shown to reflect accurately blood flow in the distal skin regions regulated by anastomosis arteriovenosa (Rubinstein and Sessler, 1990), and it provides an indirect index of distal heat loss (Krauchi *et al*, 1999). The present data with a high time resolution of 20 min have strongly confirmed previously reported hypothermic actions of Bz agonists in humans (Gilbert *et al*, 2000, 1999; Matsukawa *et al*, 1997; Pleuvry *et al*, 1980) and animals (Jackson and Nutt, 1992; Zarrindast and Dibayan, 1989).

In this study, however, we could not detect a significant thermoregulatory effect after the administration of DZP at a dose of 5 mg in spite of its clinically established sedative/hypnotic effects. One explanation is that the present subjects might possess relatively high tolerance to Bz because 5 mg of DZP showed a weak thermoregulatory effect but no significant subjective sleepiness compared to the placebo condition in these subjects. The second possibility is that there is a threshold for the plasma concentration of DZP to cause an obvious heat loss reaction in humans. Profiles of plasma DZP concentrations showed clear dose-dependent pharmacokinetics between the 5- and 10-mg DZP experiments. As a result, the average peak concentration of DZP at 5-mg experiment was 178.1 ± 11.2 ng/ml and it appeared 60 min after administration. This only slightly exceeded the lowest three measures appearing from 160 min (176.0 ± 12.8 ng/ml) to 240 min (137.6 ± 9.37 ng/ml) in the 10-mg experiment (Figure 2d). Given that a threshold exists around these plasma levels, 5 mg of DZP induced only a slight or no thermoregulatory effect. It is also possible that another Bz

agonist has its own plasma concentration threshold for inducing the heat loss reaction.

We observed a strong positive correlation between plasma DZP concentrations and the increase in DPG, indicating that DZP promotes the heat loss from distal skin regions in a dose-dependent manner. While physiological mechanistic details of heat loss action induced by Bz remain unclear, two possibilities have been given, namely involvement of the central and/or peripheral $GABA_A$ receptor. Previous studies based on receptor autoradiographic techniques have revealed that central $GABA_A$ receptors are densely localized in the thermoregulatory center including the diagonal band, medial, and lateral preoptic area in the human brain (Najimi *et al*, 1999; Zezula *et al*, 1988). There is growing evidence that thermosensitive neurons in the preoptic area and anterior hypothalamic region play a critical role in physiological sleep and the thermoregulatory response (see the review by Szymusiak *et al*, 2001). In addition, microinjection of Bz agonist or antagonist into this region has been shown to produce sleep-modifying and thermoregulatory effects in rats (Ali *et al*, 1999; Jha *et al*, 2001; Mendelson and Martin, 1992; Osborne *et al*, 1994), suggesting the possible site of action of Bz agonists through the central $GABA_A$ receptor to produce sleep-induction and hypothermic effects. Another series of studies indicated that the peripheral Bz receptor might directly cause distal heat loss. Existence of the peripheral Bz receptor in vascular smooth muscle has been shown (Cox *et al*, 1991). Although the precise function of this receptor is not fully understood (Casellas *et al*, 2002), previous *in vivo* and *in vitro* studies showed that Bz agonists can directly cause peripheral vasodilatation in animals (Barker and Clanachan, 1982; Clark and Lipton, 1981; Erne *et al*, 1989; Seubert *et al*, 2000). HR after DZP administration can inform us indirectly about this issue. Previous studies examining the Bz effect on HR, a marker of heat production in humans (Gilbert *et al*, 1999; Krauchi *et al*, 1997; Krauchi and Wirz-Justice, 1994), have yielded inconsistent findings (Ford *et al*, 1990; Matejcek *et al*, 1983 *vs* Gilbert *et al*, 1999; Pleuvry *et al*, 1980) related to HR after administration of temazepam, a clinically used Bz hypnotic. We observed a statistically significant dose-dependent increase in HR after DZP administration in subjects taking the 10-mg dose. These changes in HR after DZP administration seem to reflect the compensatory heat production for the downward deviation from the core BT set point (Kobayashi, 1989) because of direct peripheral vasodilatation by DZP rather than the sequence of heat loss promoted by a decreased BT set point driven by the thermoregulatory center, which is usually associated with suppressed heat production (Krauchi and Wirz-Justice, 1994).

There is growing evidence that the sleep-producing action in humans is commonly preceded by an increase in heat loss (Campbell and Broughton, 1994; Gilbert *et al*, 1999; Krauchi *et al*, 1999; Murphy and Campbell, 1997; Van den Heuvel *et al*, 1998). It is well established that human proximal BT declines during the night time under physiological conditions, and this is not the result of the sleep itself; it is controlled by the circadian time-keeping system (Campbell and Broughton, 1994; Gillberg and Akerstedt, 1982; Lack and Lushington, 1996; Zulley *et al*, 1981). Sleep challenge tests under isolated as well as entrained conditions reveal that the occurrence and continuity of human sleep is intimately related to the circadian proximal BT rhythms (Czeisler *et al*, 1980; Lavie, 1986; Zulley *et al*, 1981), sleep propensity increases on the falling limb, and decreases on the rising limb of the proximal BT curve. In addition, many previous studies have consistently shown that various drug-based, light exposure-based, or passive body heating-based techniques produce sleep work via thermoregulatory actions (Bunnell *et al*, 1988; Deacon *et al*, 1994; Dorsey *et al*, 1999; Gilbert *et al*, 1999; Jordan *et al*, 1990; Krauchi *et al*, 1999). They have also shown an intimate temporal relation between increasing sleepiness and the degree of heat loss induced by thermoregulatory manipulations (Gilbert *et al*, 1999; Krauchi *et al*, 1999). Krauchi *et al*, applied DPG as an indicator of distal heat loss and showed that it successfully predicts the reduction in sleep onset latency induced by various sleep-promoting modifications. Subjects with higher DPG values just before sleep had shorter sleep latencies regardless of the type of manipulation (Krauchi *et al*, 1999). Specifically, heat loss from distal skin regions could be a strong sleepiness-inducing process in humans. We too found a strong relation between DPG and two measures of subjective sleepiness after DZP administration. Our findings of a DZP concentration-dependent heat loss reaction and a heat loss-dependent increase in sleepiness lead us to believe that the heat loss process plays an important role in the onset of sleepiness after DZP administration. The findings that DZP at 5 mg showed only slight heat loss and not significant subjective sleepiness in young healthy individuals support this assumption.

The present study has also shown that the heat loss process is intimately related to the decline that occurs in psychomotor performance after DZP administration. That increased physiological sleepiness can cause impaired psychomotor performance has been shown in human extended-wakefulness studies (De Gennaro *et al*, 2001; Nakano *et al*, 2000; Thomas *et al*, 2000), shift-work model (Porcu *et al*, 1998), a clinicopathologic survey for disorders characterized by excessive daytime sleepiness (Cohen-Zion *et al*, 2001; Hood and Bruck, 1996; Kinnari *et al*, 2000; Schulz *et al*, 1997), and a clinicopharmacological survey of alertness-modifying drugs such as caffeine, modafinil, and melatonin (De Valck and Cluydts, 2001; Nave *et al*, 2002; Wesensten *et al*, 2002). Although it remains unclear whether impaired psychomotor performance after DZP administration could also be caused by increased sleepiness, the magnitude of heat loss induced by Bz agonist may be a potent indicator to predict sedative effects on brain function. Given this is the case, it is possible that Bz agonist possessing more robust hypothermic properties shows more prominent sleep-induction effects and impairment of psychomotor performances. Future study should verify that there exists not only an associative but also a causative link between heat loss and brain functions after DZP administration, in which, for example, whether prevention of the heat loss induced by Bz results in an absence or diminution of sedative/hypnotic effects should be examined.

In spite of the many studies, the physiological processes of Bz action through $GABA_A$ receptor remain poorly understood. One reason is that the GABAergic projection

is the most widespread of the inhibitory neurotransmitters and could affect many circuits that are responsive to the various effects of Bz agonists. In addition, there exist various subtypes of $GABA_A$ receptor showing different affinity and channel functions in response to Bz agonists and possessing various combinations of plural types of alpha-, beta-, and gamma-subunits (Barnard *et al*, 1998; McKernan and Whiting, 1996). These subtypes of $GABA_A$ receptor broadly distribute in various brain circuits to form an overlapping mosaic of receptor subtypes (Wisden and Stephens, 1999), making it difficult to understand the functional mechanisms of Bz agonists through the $GABA_A$ system from the physiological point of view (Rudolph *et al*, 1999). The thermoregulatory effects observed in the present and previous studies offer important information about the physiological pathway of sedative/hypnotic actions induced by Bz agonists and could be useful physiological markers for future studies.

ACKNOWLEDGMENTS

The present study was performed through Special Coordination Funds of the Ministry of Education, Culture, Sports and Technology, and a Grant-in-Aid for Cooperative Research from the Ministry of Health, Labour and Welfare of Japan.

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