NOTE
Nocturnal Enhancement of Plasma Melatonin Could Be Suppressed by Benzodiazepines in Humans

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Abstract

Plasma melatonin levels were determined every 20 and 30 min for 24 hours on the last day of repeated oral administrations (1 or 2 mg a day for 8 or 9 days) of a benzodiazepine derivative (450191-s), which is known to be metabolized to active benzodiazepines after administration. In one of the two subjects, the nocturnal enhancement of plasma melatonin which was obvious on a control day with placebo was diminished almost completely. In the other subject, observed were not only the diminishment of its nocturnal enhancement but also its increase during the daytime almost to the nocturnal levels on a control day, which may indicate a rebound increase in melatonin synthesis or a shift in its day-night rhythmicity.

Such suppressing effects of benzodiazepines on the nocturnal plasma melatonin levels were also examined in the case of a single administration of 2 mg of 450191-s or flunitrazepam in the second series of experiments. Even a single flunitrazepam seemed to have lowered nocturnal plasma melatonin levels, which then recovered to the usual levels following the administration of 5 mg of a benzodiazepine antagonist, Ro 15-1788, given 6 hours after the flunitrazepam. However, single 450191-s did not show any remarkable effects.

Thus, it has been suggested that benzodiazepines could suppress the nocturnal levels of plasma melatonin or shift its day-night rhythmicity at least when administered repeatedly. The possible action site of benzodiazepines may be the central nervous system, since melatonin synthesis has been thought to be under strongly regulated by the central nervous pathway from the retina to the pineal body. Therefore, these effects of benzodiazepines may provide a method for investigating the physiological role of melatonin and its day-night rhythmicity as well as to further clarify the system regulating melatonin synthesis in humans.

Pineal melatonin synthesis has been thought to be regulated predominantly by the central neuronal pathway from the retina to the pineal beta-adrenergic receptors (Axelrod, 1978; Klein, 1978; Wurtman, 1970, 1980), while peripheral catecholamines.
have been shown to increase it but slightly in some stress conditions (Lynch et al., 1977). However, it has not yet been fully understood what synapses are included in that pathway or what kinds of other neurons could indirectly affect the pineal melatonin synthesis via that pathway. In the present experiments, the possibility of the involvement of a benzodiazepine-GABA receptor complex in the brain has been examined preliminarily. Melatonin and its metabolite in the brain, N-acetyl-5-methoxy-kynurenamine, on the other hand, have been shown to bind strongly and competitively with diazepam to the brain benzodiazepine receptors (Green et al., 1981; Marangos et al., 1981). The effects of benzodiazepines on human growth hormone (hGH) and prolactin (hPRL) were also examined simultaneously, since hGH secretion stimulated by clonidine has been reported to be suppressed by diazepam (Koulu et al., 1983).

Materials and Methods

The Subjects were 5 healthy male students aged 20 to 22 years. Experiments consisted of two parts to see the chronic effects of repeated administrations of a new benzodiazepine derivative, 450191-s (Shionogi Seiyaku K. K.), and acute effects of a single administration of 450191-s or flunitrazepam, each of which was followed by a benzodiazepine antagonist, Ro15-1788 (Hoffman-La Roche), 6 hours after it. The light in the experimental room was turned off at 2300 hours and on at 0800 hours in the next morning in all the experiments. 450191-s has been shown to be metabolized to active benzodiazepines after administration (unpublished data by Shionogi Seiyaku K. K.).

i) Chronic Experiments

Two or 1 mg of 450191-s was orally administered every day at 2200 hours for 9 or 8 days successively to Subjects 1 and 2, respectively. On the last day of its administrations, blood samplings were started about 1 or 2 hours before giving the last 450191-s and continued every 30 min during the light period and 20 min during the dark period for 24 hours, through an indwelling cannula kept in the vena mediana cubiti. As for Subject 1, on the same schedule as above, blood samples were collected for a control day when a placebo was given instead of 450191-s.

ii) Acute Experiments

Under the same lighting and blood sampling schedules, Subject 3 was given orally 10 mg of Ro 15–1788 at 0400 hours, Subject 4 was given 2 mg of 450191-s at 2200 hours and 5 mg of Ro 15–1788 6 hours later or at 0400 hours, and Subject 5 was given 2 mg of flunitrazepam at 22 hours and 10 mg of Ro 15–1788 6 hours later.

In both of the above experiments, the sleep polygram was recorded simultaneously with blood samplings and sleep stages were analysed according to the criteria by APSS (Rechtschaffen and Kales, 1968).

Plasma melatonin, hGH and hPRL were determined by radioimmunoassay methods. However, melatonin was extracted before assay according to Lang et al.'s method (1981) with slight modifications. The antisera for melatonin was kindly donated by Dr. Levine (Brandeis University, MA).

Results

i) Chronic Effects of 450191-s on Melatonin

In Subject 1, melatonin did not show any enhancement on the last day of 450191-s administrations (Fig. 1-b), but was observed on the placebo control day (Fig. 1-a). Similar suppressing effects of repeatedly administered 450191-s were also obvious in Subject 2 (Fig. 2). The daytime levels of plasma melatonin in Subject 2, however, were increased markedly.

ii) Acute Effects of 450191-s or Flunitrazepam on Melatonin

As shown in Fig. 3 for Subject 3, no apparent effect on plasma melatonin of only a benzodiazepine antagonist, Ro15–1788, at a dose of 10 mg was found. However, 5 mg of it seems to have suppressed melatonin levels when it was given 6 hours after 2 mg
Fig. 1-a. Plasma melatonin, PRL and hGH levels throughout the control day of Subject 1 when placebo was given orally at 2200 hours.
Fig. 1-b. Plasma melatonin, PRL and hGH levels throughout the last day of repeated administrations of 2 mg of 450191-s daily for 9 days in Subject 1 are shown. Arrows show the time of the last administration of 450191-s or 22 hours.
Fig. 2. Plasma melatonin, PRL and hGH levels throughout the last day of repeated administrations of 1 mg of 450191-s daily for 8 days in Subject 2 are shown. As for the arrow, see Fig. 1-b.
Fig. 3. Plasma melatonin, PRL and hGH levels throughout 24 hours on the day when 10 mg of Ro15-1788 was given orally at 0400 hours in Subject 3 are shown. Arrows indicate the time of the drug administration.
Fig. 4. Plasma melatonin, PRL and hGH levels throughout 24 hours on the day when 2 mg of 450191-s was given at 2200 hours and 5 mg of Ro1501788 was also administered 6 hours later or 0400 hours in Subject 4 are shown. As for the arrows, see Fig. 3.
Fig. 5. Plasma melatonin, PRL and hGH levels throughout 24 hours on the day when 2 mg of flunitrazepam was administered at 2200 hours and 10 mg of Ro15-1788 was also given 6 hours later or at 0400 hours in Subject 5 are shown. As for the arrows, see Fig. 3.
of 450191-s as shown in Fig. 4 for Subject 4, though 2 mg of 450191-s itself did not show any effect on melatonin.

Two mg of flunitrazepam, on the other hand, may have inhibited the nocturnal enhancement of melatonin levels to its daytime ones. This inhibiting effect, however, seems to have been antagononized by a subsequent administration of 10 mg of the antagonist.

**iii) Effects of 450191-s or Flunitrazepam on plasma hGH and hPRL**

As shown in Fig. 1-b, 2, 3 and 4, no apparent effects of 450191-s or flunitrazepam on plasma PRL and hGH were detected in either the chronic or the acute experiments. The antagonist, Ro 15–1788, itself showed no apparent effects either.

**Discussion**

The suppressing effect of repeatedly administered 450191-s on the nocturnal enhancement of plasma melatonin levels has been strongly suggested by the present preliminary study. In the case of single administration, only flunitrazepam may also have produced such a suppressing effect as the above, which seems to have been antagonized by Ro15–1788, the most specific one of the known antagonists for benzodiazepine receptors.

The daytime increase in plasma melatonin, with the nighttime levels being suppressed in Subject 2 repeatedly given 1 mg of 450191-s, showed the rebound of melatonin synthesis in response to its suppression during the preceding nights, or its day-night rhythmicity may have been shifted almost completely as a chronic effect of 450191-s. In either case, however, this daytime increase in plasma melatonin should also be examined further in relation to the doses of 450191-s as well as to the individual difference in susceptibility to the drug.

On the other hand, a single administration of 450191-s does not seem to have affected the plasma levels of melatonin. Ro 15–1788 which was given after the 450191-s even suppressed the melatonin level to its daytime level, while Ro 15–1788 itself when administered singly did not show any effect on the level. This contradictory effect of the antagonist might be attributed to the characteristics of the drug used or to the awakening effect of the antagonist itself, which should also be clarified further. In relation to the former possibility, it should be noted that 450191-s is known to have weaker activity in inducing muscle relaxation or anti-convulsion than flunitrazepam or flurazepam. Also as for the latter possibility, Ro15-1788 was thought to have produced a strong awakening effect by antagonizing the sedative effects of the benzodiazepines as observed on the EEG records.

Thus, at least chronic treatment with benzodiazepines may provide a method to investigate further the neuronal regulating system of melatonin synthesis in humans. Also the possibility of melatonin involvement in benzodiazepines exerting their effects as hypnotic or anti-anxiety drugs should be examined further, and this might clarify the physiological role of melatonin or its rhythmicity. The diminished melatonin secretion, especially during night time, has so far been related to depression (Wetterberg, 1983), aging (Iguchi, 1981; Reiter et al., 1980), or senile dementia of the Alzheimer type (Kabuto et al., 1982).

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References


