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Effects of Methylphenidate on Oxytocin and Vasopressin Levels in Pinealectomized Rats During Light–Dark Cycle

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APPENRODT, E., E. BOJANOWSKA, J. JANUS, B. STEMPNIAK, J. W. GUZEK AND H. SCHWARZBERG *Effects of methylphenidate on central and and peripheral oxytocin and vasopressin levels in pinealectomized rats during light–dark cycle.* PHARMACOL BIOCHEM BEHAV **58**(2) 415–419, 1997.—Although previous reports have shown that methylphenidate (MPH), in addition to its known behavioral effect, can influence the hypothalamo–pituitary–adrenal axis by increasing the plasma ACTH, the pineal gland seems to be involved in neuroendocrinological processes too, e.g., in hypothalamic synthesis and release of oxytocin (OXY) and vasopressin (AVP). Therefore, a study was performed to measure the OXY and AVP content of the hypothalamus, neurohypophysis, and plasma after application of MPH in the morning and evening in pinealectomized (PE) as well as sham-operated control (SO) rats. Pinealectomy influenced both the daily pattern (reversed in the neurohypophysis) and the levels of OXY and AVP. Starting from this different situation, application of MPH produced diverse effects. Hypothalamus: PE, increase in both hormones in the morning and evening; SO, decrease in morning OXY level. Neurohypophysis: PE, increase in morning OXY level; SO, decrease in both hormones even though in the morning only. Plasma: PE, decrease in morning OXY concentration; SO, increase in both hormones in the morning and decrease in the evening. The present results indicate that MPH application influences the hypothalamo–neurohypophysial system. Furthermore, the hypothesis has been supported that this influence may be dependent on the circadian activity of the pineal gland as well. © 1997 Elsevier Science Inc.

Methylphenidate Pineal gland Oxytocin Vasopressin Rats

THE hypothalamo–pituitary–adrenal axis is influenced by a number of neurotransmitter systems such as catecholamines, serotonin, GABA, and acetylcholine (6,13,26). A great many findings have suggested that the pineal gland, too, with its light–dark-dependent activity can influence this axis (8,24), transmitter systems (1,2,3), and behavior (20,23). It has been demonstrated that vasopressin (AVP) and oxytocin (OXY) levels in brain and plasma exhibit marked changes during the light–dark cycle, and pinealectomy as well as constant light were found to affect these cyclic changes (33).

The psychostimulant, methylphenidate (MPH) is known to produce a dose-dependent increase in the dopamine (DA) level in the brain by inhibition attributed to DA uptake (7,15). This effect is associated with specific behavioral reactions, e.g., enhanced locomotor activity (4,25). It has been shown that MPH can induce a circadian locomotor rhythm after it has been abolished (14).

Hence, it is desirable to investigate whether or not a psychostimulant such as MPH is capable of influencing the hypothalamo–neurohypophysial system as a function of its interaction with the pineal gland. Therefore, a series of experiments was conducted to measure the OXY and the AVP content of the hypothalamus, neurohypophysis, and plasma after application of MPH in the morning and in the evening in pinealectomized as well as in sham-operated rats.

METHODS

The experiments were performed in male Wistar rats weighing 190–250 g. The animals were kept under a 12:12-h

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light:dark cycle (light on at 0600). Food and water were available ad lib.

The animals were pinealectomized or sham-operated under hexobarbital anaesthesia (0.2 g/kg body weight). For more detailed information about the specific surgical procedure, see (32). The final experiments were conducted after a recovery period of two weeks.

Each of the pinealectomized as well as the sham-operated series were divided into two groups. In the "morning group," drug application started at 0630, i.e., after the end of the darkness period, and in the "evening group" at 1730, i.e., toward the end of the light period. Pinealectomized as well as sham-operated animals were given an IP injection of either MPH (Ritalin, CIBA-GEIGY, 50 mg/kg body weight) or 100 μl NaCl solution (0.154 M).

The rats were decapitated 30 min after drug application. The brains were removed immediately and dissected to obtain neurohypophysis and hypothalamus and to confirm the presence or absence of the pineal gland. Plasma samples were obtained from collected trunk blood. OXY and AVP were extracted from plasma using C18 Sep-Pak columns (Waters Associates Ltd., Northwick, U.K.), and from the tissue using 0.2 and 0.4 mmol/l acetic acid, respectively, as described (11). Samples were stored at −20°C until hormone levels were determined by radioimmunoassay (RIA).

The cross reactivity of the AVP RIA with other related peptides including OXY was less than 1%, and, in the OXY RIA, 1.12% cross reactivity with AVP. The detection limit of the AVP RIA was 1.73 pg per tube with intra- and interassay variation coefficients of 3.9% and 6.3%, respectively. The corresponding values for the OXY RIA were 3.56 pg per tube, 4.9% and 9.1%. The OXY/AVP concentrations in tissue extracts as well as plasma were expressed as nanograms per whole hypothalamus/neurohypophysis or picograms per milliliter blood plasma.

The data were presented as mean values ± SE. For statistical evaluation, a three-way analysis of variance with GLM procedure (SAS/STAT Software, SAS Institute Inc., Cary, N.C., 1989) was used; the factors were: operation (sham-operated or pinealectomized), time (morning or evening), and treatment (MPH or NaCl). A level of $p < 0.05$ was considered as statistically significant. For further interpretation, Student's *t*-test was employed for individual vs. group comparisons.

RESULTS

The IP application of 50 mg/kg MPH induced remarkable stereotype behavioral reactions, such as enhanced locomotor activity (in the first minutes), licking, sniffing, and later gnawing. The reactions started approximately 10 min after application with an intensity peak after 30 min, and finished after about 2 h.

Content of OXY and AVP in the Hypothalamus

As shown in Fig. 1, no differences were seen between morning and evening values in sham-operated and in pinealectomized animals. Compared with sham-operated animals, pinealectomy diminished the hypothalamic AVP content in the morning; however, no difference was observed in the evening values. No such differences were noted in the OXY content.

After application of MPH in sham-operated animals, the morning OXY content only was decreased, whereas the other values exhibited no changes. In pinealectomized animals, an increase in both OXY and AVP was seen in the morning and evening values; the difference in the morning OXY values only was not clearly statistically significant.

Content of OXY and AVP in the Neurohypophysis

The results are summarized in Fig. 2. The content of both hormones in sham-operated animals was higher in the morning than in the evening. Also, the pinealectomized animals showed statistically significant differences between morning and evening levels of both OXY and AVP, even though morning values were lower and evening levels higher. The morning levels of both hormones were found to be significantly higher in the sham-operated than in the pinealectomized animals, but no differences were found between the evening values.

Following application of MPH in sham-operated rats, morning OXY and AVP levels were diminished; however, MPH was without effect in the evening. In pinealectomized rats, the morning OXY values were increased after MPH, but neither the evening OXY content nor the AVP content in the morning or evening were influenced.

Plasma Concentration of OXY and AVP

The changes in plasma OXY and AVP levels are shown in Fig. 3. In sham-operated animals, the concentration of both hormones was lower in the morning than in the evening. In pinealectomized animals, no significant differences of OXY and AVP concentrations were seen between morning and evening values. However, it could be demonstrated that, in the pinealectomized rats, the morning concentration of both OXY and AVP was significantly higher than in sham-operated rats (see above), whereas no differences were found between the evening values.

Following injection of MPH, diverse reactions were noted. An increase in morning levels of both OXY and AVP were found in sham-operated animals; the evening concentrations, however, were seen to be diminished, even though the latter finding was not statistically significant. No such reaction was observed in the pinealectomized animals. In these animals, the OXY concentration decreased after MPH application in the morning, whereas no effect was noted in the evening. The AVP concentration was not affected in the morning and in the evening.

DISCUSSION

The enhanced locomotor activity and the specific stereotype behavior after application of MPH as seen in the present experiments are consistent with results of other workers (30). This behavior can be inhibited partially or completely by various neuroleptics in a dose-dependent manner, but they differed markedly in their effects (19). Because approximately 30 min after injection of the drug the change in behavior was very pronounced, this time point was chosen to analyze the OXY and AVP content in brain and plasma.

It is described in the literature that MPH influences not only the DA content in the brain (21,34), but also the hypothalamic–pituitary–adrenal axis in that it increases the plasma ACTH and corticosterone levels (18,27,29). We believed that MPH may cause functional changes in the hypothalamo–neurohypophysial axis too, because there are relationships between the two axes, e.g., AVP acts as an important ACTH secretagogue (17,22). This supposition has been supported by the present findings; it was noted that MPH exerted marked

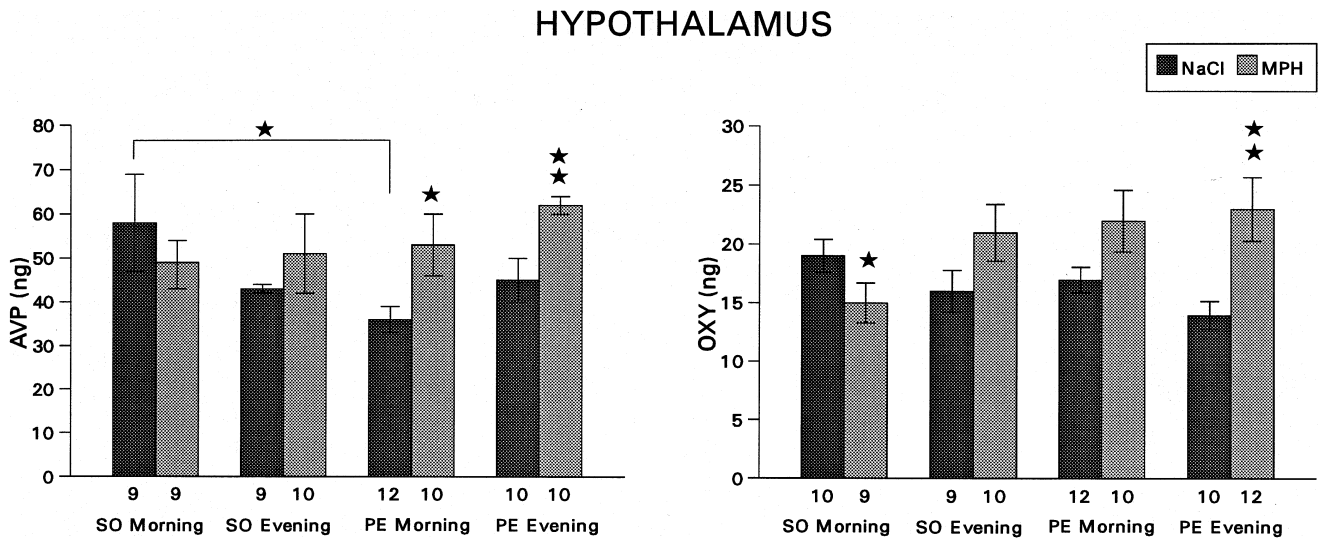

FIG. 1. Effects of methylphenidate on oxytocin (OXY) and vasopressin (AVP) content in the hypothalamus of sham-operated (SO) and pinealectomized (PE) rats. MPH, 50 mg/kg methylphenidate; NaCl, 100 μl sodium chloride solution. The number of animals of each group is indicated below the respective bar. Mean values ± SE: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

effects on AVP and OXY levels in plasma, neurohypophysis, and hypothalamus.

OXY and AVP show a marked 24-h cycle pattern (33). The significant increase in both hormones in the plasma during the day and the simultaneous decrease in the neurohypophysis were also found by other workers (10). However, in the hypothalamus, such variations between morning and evening values have not always been so pronounced and the results are particularly contradictory (16,31).

Interestingly, the effects of MPH on OXY and AVP levels were different in sham-operated and pinealectomized rats. It has been suggested that the pineal gland is involved in the regulation of many physiological rhythms, including those of synthesis and release of pituitary hormones [for review see: (28)]. Apparently, the reaction of MPH on OXY and AVP levels (i.e., increase, decrease, or without effects) depends on pineal-influenced circadian basic levels of both hormones.

After pinealectomy, this variability seemed to be relatively restricted; in the hypothalamus alone, we found changes in hormone content comparable with those in sham-operated animals. Results indicating that amphetamine increased the ACTH and corticosterone levels but failed to influence the plasma concentration of AVP (30) should be looked at from the circadian viewpoint. We found highly significant effects on plasma AVP level, even though in the morning only.

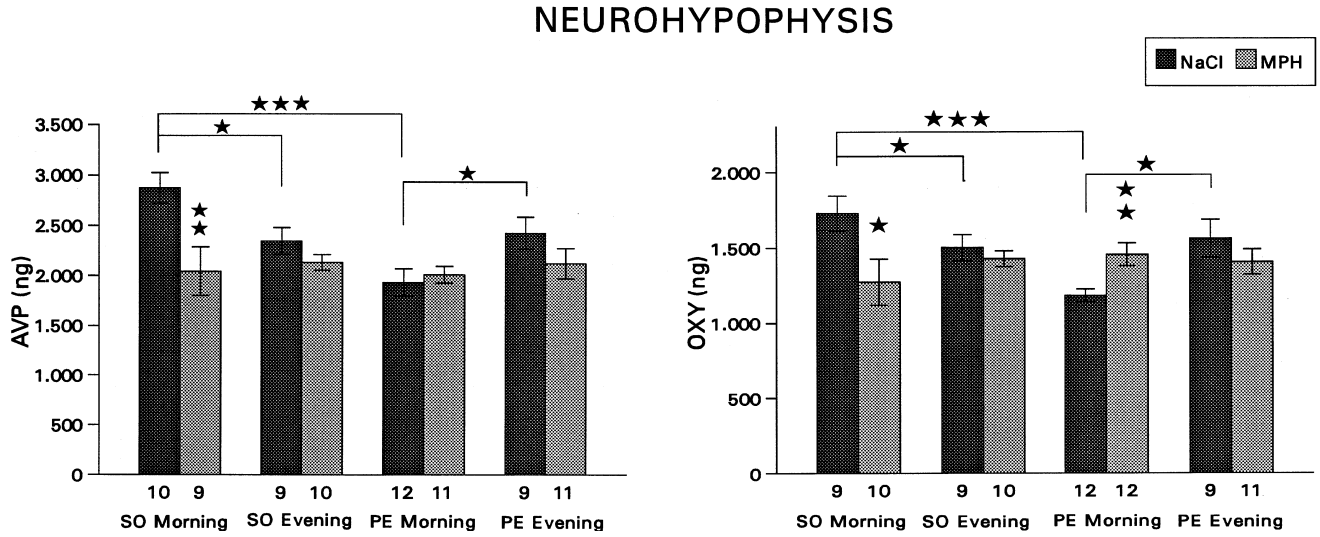

FIG. 2. Effects of methylphenidate on oxytocin (OXY) and vasopressin (AVP) content in the neurohypophysis of sham-operated (SO) and pinealectomized (PE) rats. MPH, 50 mg/kg methylphenidate; NaCl, 100 μl sodium chloride solution. The number of animals of each group is indicated below the respective bar. Mean values ± SE: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

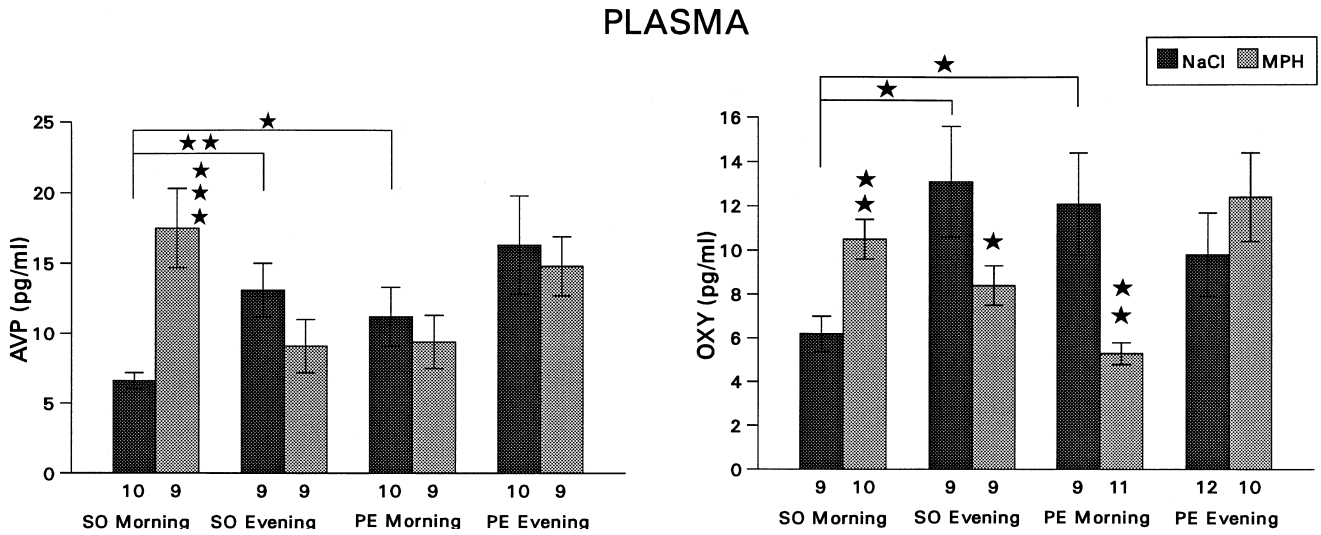

FIG. 3. Effects of methylphenidate on oxytocin (OXY) and vasopressin (AVP) content in the blood plasma of sham-operated (SO) and pinealectomized (PE) rats. MPH, 50 mg/kg methylphenidate; NaCl, 100 μl sodium chloride solution. The number of animals of each group is indicated below the respective bar. Mean values ± SE: $*p < 0.05$, $**p < 0.01$.

The present results indicate that the pineal can influence both the daily pattern and the levels of OXY and AVP. Our finding that, after pinealectomy, the pattern of neurohypophysial hormone content with the normal decrease over the day was reversed is consistent with an observation of other workers (12). However, we did not find a clearly reversed pattern in the periphery. Altered hormone levels after pinealectomy, i.e., higher plasma and lower neurohypophysial values were also found by other workers (9).

On the one hand, the present data indicate that MPH application can influence the hypothalamo–neurohypophysial system, and the pineal gland seems to be capable of modulating this influence. On the other hand, MPH and other psychostimulants producing similar effects (e.g., amphetamine) are known to influence behavior (5). It has to be clarified in further investigations whether the changes in OXY and AVP levels after MPH application may, at least partly, modulate such behavioral reactions.

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