Psychoneuroendocrinology, Vol. 17, No. 6, pp. 611–617, 1992
Printed in Great Britain

0306–4530/92 $5.00+0.00

APOMORPHINE STIMULATION OF VASOPRESSIN- AND OXYTOCIN-NEUROPHYSINS. EVIDENCE FOR INCREASED OXYTOCINERGIC AND DECREASED VASOPRESSINERGIC FUNCTION IN SCHIZOPHRENICS

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(Received 26 April 1991; in final form 24 October 1991)

SUMMARY

Apomorphine challenge tests (0.5 mg SC) were performed in 14 normal male volunteers and in 9 male schizophrenic inpatients, drug-free for at least 2 wk. In the normal volunteers, apomorphine induced an increase of serum growth hormone (GH) (maximum at 40 min), of vasopressin-neurophysin (hN_PI) (maximum at 20 min), and oxytocin-neurophysin (hN_PII) (maximum at 20 min). The release of neurophysins was independent of digestive side effects. In the schizophrenics, the GH level and release pattern were similar to those in the controls. The basal level of hN_PI was reduced (t_0: 0.42±0.1 ng/ml in the schizophrenics and 0.66±0.05 ng/ml in the controls, $p<0.02$). In contrast, the basal level of hN_PII was increased (3.34±0.04 ng/ml in the schizophrenics to 0.92±0.21 ng/ml in the controls, $p=0.001$). The response to apomorphine was blunted, with no significant release of hN_PI or of hN_PII. Although the hN_PII data are consistent with an increased dopaminergic tone, the psychopathological meaning of the increased basal oxytocinergic and decreased vasopressinergic functions remains to be defined.

INTRODUCTION

IT HAS BEEN well demonstrated that the dopamine agonist apomorphine stimulates the release of various anterior pituitary hormones: mainly growth hormone (GH) (Brown *et al.*, 1974), but also ACTH, cortisol, and β-endorphin (Jezova & Vigas, 1988). The GH-releasing activity of apomorphine has been largely used in endocrinology to test the integrity of the anterior pituitary, but also in biological psychiatry to test the sensitivity of brain hypothalamic dopamine receptors (Ansseau *et al.*, 1987).

Dopamine also has been reported to influence the secretion of posterior pituitary hormones. However stimulatory (Moos & Richard, 1982; Bridges *et al.*, 1976; Kimura *et al.*, 1981) as well as inhibitory (Forsling *et al.*, 1981) actions on vasopressin (AVP) secretion have been described in animals. Similar controversies exist concerning oxytocin (OT) release since both

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stimulatory (Moos & Richard, 1982) and inhibitory (Seybold *et al.*, 1978; Vizi & Volbekas, 1980) actions have been described.

Few studies have investigated the influence of dopamine agonists and antagonists on AVP in humans. Lightman and Forsling (1980) reported that L-dopa inhibits its resting levels. Influence of dopamine antagonists is controversial: Haloperidol and metoclopramide were reported to have either no effect (Kendler *et al.*, 1978) or a stimulatory one (Nomura *et al.*, 1984) on AVP release.

We previously reported a stimulatory influence of acute apomorphine injection on AVP neurophysin release in one manic patient (Legros & Ansseau, 1986); Nussey *et al.* (1988) also described a stimulatory action of apomorphine on AVP and OT in the human. In the present study, we tested the influence of apomorphine injection on plasma AVP- and OT-neurophysin levels (a direct index of posterior pituitary activation) (Legros, 1975) in a group of normal subjects and compared basal levels and the patterns of response to those observed in a group of schizophrenic patients of similar age. Dopamine dysfunction has been widely suggested in schizophrenic, and the neurophysin response to apomorphine challenge could represent an indirect way to test this hypothesis.

SUBJECTS AND METHODS

Subjects

The apomorphine stimulation test was conducted as previously described (Legros & Ansseau, 1986) in 14 normal male volunteers without history of drug addiction and who were nonsmokers (mean [±SEM] age, 24.3±0.9 yr) and in 9 male schizophrenic inpatients (mean [±SEM] age, 28.4±3.5 yr). There was no significant difference between the mean age of these two groups ($F_{1,21}=1.91$, $p<0.20$). All patients met Research Diagnostic Criteria (RDC) for schizophrenic (Spitzer *et al.*, 1978; Ansseau, 1985) and were tested after a drug-washout period of at least 2 wk. Four schizophrenics had never received neuroleptics, and none of the patients had been treated with long-acting preparations. Volunteers were tested in the outpatient clinic of the Neurophysiological Laboratory. All subjects were free of medical illness as evidenced by history and medical examination. The protocol of this study was approved by the Ethical Committee of the University of Liége Medical School, and all subjects gave their informed consent.

Procedure

In the fasting recumbent subjects, an intracatheter was placed in the forearm at about 0830h. After 30 min rest, a first blood sample (about 10 cc) was taken (time -20 min) and a second one 20 min later (time 0). Apomorphine (0.5 mg SC) was injected, and blood was taken 20, 40, 60, and 120 min after injection. After each blood sampling, side-effects were carefully rated as follows: 0, no subjective side-effects; +, nausea without vomiting; ++, nausea and vomiting.

GH was measured with a double antibody radioimmunoassay (RIA) (Franchimont, 1968), with intra- and interassay coefficients of variation of 13.3% and 14.8%, respectively, and a detection limit of 0.2 ng/ml. AVP-neurophysin (hN_PI) was assayed by RIA as previously described (Legros & Ansseau, 1986), with intra- and inter-assay coefficients of variation of 6.5% and 10.7%, respectively, and a detection limit of 0.1 ng/ml. OT-neurophysin (hN_PII) was directly estimated by subtracting hN_PI from total immunoreactive neurophysins (IRN) (Legros *et al.*, 1969), as described previously (Scott *et al.*, 1989).

Data analysis

Basal values were compared between normal male volunteers and schizophrenic patients by a one-way analysis of variance (ANOVA). The significance of release was tested between groups by analysis of variance with repeated measures. Correlations (Spearman) between releases were calculated using the relative area under the curve (AUC). Results are expressed as mean±SEM for each time. The level of significance was set at 0.05. All statistical tests are two-tailed.

Since it appeared that the variation in neurophysin release was greater in the patients than in the controls (cf.

Results), we also assessed the influence of several demographical and clinical parameters (age, duration of episode, BPRS score) using Spearman correlation coefficients. Finally, we compared two RDC clinical subtypes of schizophrenia defined as paranoid (n=4) and non-paranoid (catatonic, n=2; undifferentiated, n=1; disorganized, n=1) by Student *t*-test.

RESULTS

Mean (±SEM) GH, hN_PI and hN_PII serum levels in normal male volunteers are shown in Fig. 1. This group exhibited a clear-cut increase in GH serum concentration ($F_{5,65}=48.15$, $p<0.0001$), the maximum values being observed at +40 and +60 min. hN_PI also exhibited a highly significant, five-fold increase from a basal value (time 0) of 0.66±0.05 ng/ml to a maximum value of 2.09±0.5 ng/ml at +20 min ($F_{5,65}=8.15$, $p<0.0001$). Concomitantly, hN_PII showed a two-fold increase from a basal value of 0.92± 0.21 ng/ml to a maximum value of 2.17± 0.4 ng/ml at +20 min ($F_{5,65}=6.85$, $p<$ 0.0001).

No significant correlation was found between hN_PI and hN_PII AUC ($r=0.01$, $p=0.96$), nor between GH and hN_PI ($r=$ 0.111, $p<0.7$) or between GH and hN_PII ($r=0.4$, $p=0.12$).

Ten of fourteen normal subjects experienced nausea or vomiting beginning 10 min after apomorphine administration and lasting for about 15 min. Subjects released GH, hN_PI or hN_PII regardless of whether or not they experienced nausea or vomiting. There was no significant relationship between hN_pI ($r=0.5$, $p=$ 0.05) and hN_PII ($r=-0.04$, $p=0.98$) and the rating of digestive side-effects (Fig. 2). The GH release was highly significant ($F_{5,40}=4.33$, $p<0.003$) and similar to that observed in the normal volunteers ($F_{5,65}=48.15$, $p<0.0001$). Basal hN_PI (0.42±0.1 ng/ml) was reduced compared to the volunteers (0.66± 0.05 ng/ml) ($F_{1,21}=6.82$, $p=0.02$), and no significant release of hN_PI could be demonstrated after apomorphine injection ($F_{5,40}=1.19$, $p=0.33$).

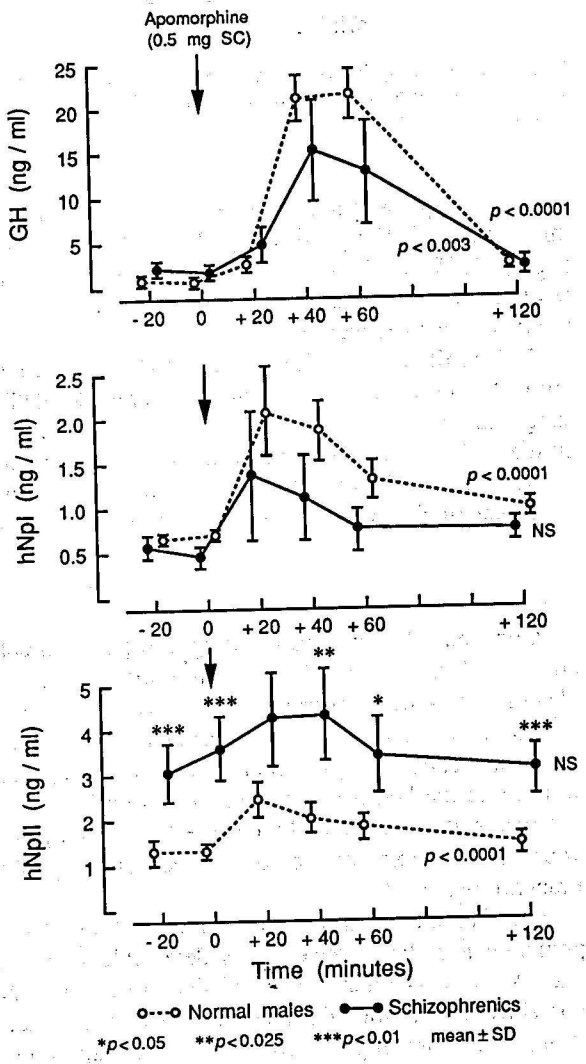

FIG. 1: Mean serum levels of GH, vasopressin-neurophysin (hN_PI) and oxytocin-neurophysin (hN_PII) in 14 male normal volunteers and 9 male schizophrenics. The significance of the response to apomorphine is given at the right of the graph. The significance of the differences between groups at different times of the test are shown as *, **, ***.

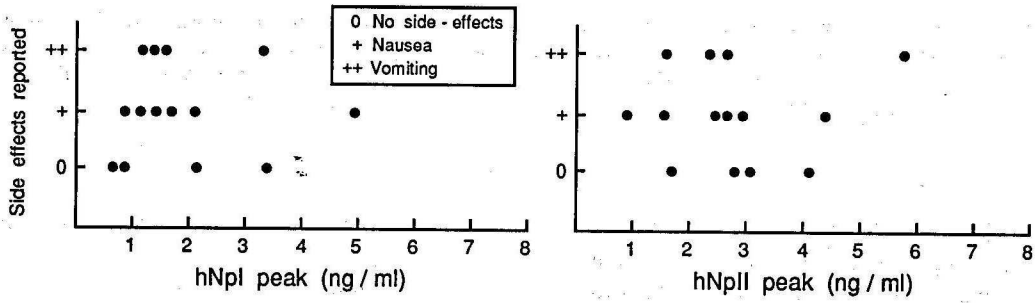

FIG. 2: Relationship between digestive side-effects (0, +, ++) and vasopressin-neurophysin (hN_PI) peak (upper part) and oxytocin-neurophysin (hN_PII) peak (lower part) in the course of the apomorphine stimulation test in 14 normal male volunteers.

Basal hN_PII was more elected in the schizophrenics than in the volunteers (3.34 ± 0.74 ng/ml vs. 0.92 ± 0.21 ng/ml, $F_{1,21} = 14$, $p < 0.001$), and no significant release of hN_PII was noted ($F_{5,40} = 1.55$, $p = 0.2$). No significant relationship existed between hN_PI and hN_PII AUCs ($r = 0.43$, $p = 0.24$), nor between GH response and either hN_PI ($r = 0.52$, $p = 0.14$) or hN_PII ($r = 0.28$, $p = 0.47$) release.

No significant relationship existed between basal and stimulated hN_PI or hN_PII and the clinical parameters (age, duration of episode, BPRS score). The paranoid and non-paranoid subgroups, which did not differ concerning age, duration of episode and BPRS score, showed similar hN_PI and hN_PII basal values and AUCs. In contrast, basal hN_PII was significantly higher in the paranoid (5.15 ± 0.99 ng/ml) than in the non-paranoid group (1.92 ± 0.61, $p < 0.03$).

Interestingly, no digestive side-effects were noticed in any of the schizophrenics.

DISCUSSION

Our study demonstrates that apomorphine injection stimulates not only GH but also hN_PI and, to a lesser extent, hN_PII in normal men. A similar stimulatory effect on AVP and OT plasma levels was observed in nine normal young subjects by Nussey *et al.* (1988). Those investigators, however, used higher doses of apomorphine (50 µg/kg SC; i.e., about 3.5 mg for an adult weight 70 kg), which presumably explains why the observed digestive side-effects were more marked (nausea in all, vomiting in seven of nine of the subjects) than in our study. Moreover, their study included five female subjects, whereas ours was conducted entirely in young men, to exclude the possible confounding effect of estrogen on hN_PII release (Legros & Franchimont, 1970).

Our data clearly show that hN_PI as well as hN_PII can be released by low doses of apomorphine, even in the absence of any detectable side-effects. The independence between vomiting and AVP and OT release was also demonstrated by Nussey *et al.* (1988), since those investigators showed that oral administration of ipecacuanha, a potent emetic drug, was responsible for nausea and vomiting of similar order of magnitude than that observed in their apomorphine protocol, but did not induce AVP or OT release.

In our study, the time course of the GH peak (+40 to +60 min) and the hN_PI and hN_PII peaks (+20 min) were slightly different, possibly explained by the fact that apomorphine stimulates GH release via its hypothalamic releasing factor (GHRH?), whereas neuropituitary peptides are directly released into peripheral circulation following dopaminergic stimulation.

The reduced basal hN_PI in the schizophrenics is consistent with previous findings on cerebrospinal fluid (CSF) (Linkowski *et al.*, 1984) and with the beneficial role of AVP on schizophrenic symptoms (mainly emotional withdrawal, anergic and blunted affect) noted in several trials (Forizs, 1952; Vranckx *et al.*, 1979; Legros & Lancranjan, 1984). Moreover, Van Kammen *et al.* (1981) described a significant decrease in CSF AVP in schizophrenics compared to healthy controls.

The increased plasma hN_PII found in the drug-free schizophrenics confirms our previous finding of increase CSF hN_PII (Linkowski *et al.*, 1984) and is consistent with another report of an increased CSF OT (Beckmann *et al.*, 1985). Those investigators demonstrated this increase in patients without any treatment as well as in patients under chronic neuroleptic treatment, the levels being slightly but significantly more elevated in the latter group. However a 3-wk haloperidol treatment did not induce any change in CSF OT concentrations (Beckmann *et al.*, 1985). In our study, two of the four drug-naïve patients exhibited high hN_PII levels, confirming the independence between neuroleptic treatment and OT levels.

Our study also suggests a decrease in the sensitivity of hN_PII to apomorphine challenge in schizophrenics. Although caution is warranted in the statistical interpretation of the data due to the small sample size, it is tempting to speculate that an increase endogenous dopaminergic tone could be responsible for the decreased sensitivity to exogenous dopaminergic agonists. The meaning of the basal increase in oxytocinergic function in schizophrenic patients observed in all three studies also must be discussed. The origin of this increase is still unknown: An activation of the endogenous dopaminergic drive can be postulated, but other hormones known to be under stimulatory dopaminergic tone, in particular GH, are not elevated in schizophrenics.

The hypothetical consequences of such an increase of oxytocinergic function are also speculative: The presence of OT receptors in the brain, particularly the hippocampus, is well demonstrated (Mühlethaler *et al.*, 1982) and could explain why OT (Bohus *et al.*, 1978) and OT fragments (Burbach *et al.*, 1983) exhibit behavioral action. In previous reports, we showed that OT infusion can alter evoked potentials in normal male volunteers (Geenen *et al.*, 1988), while OT treatment given to an obsessive-compulsive patient induced psychotic effects (delusions, hallucinations) (Ansseau *et al.*, 1987). In that respect, our preliminary finding of a higher basal hN_PII in the paranoid schizophrenics could suggest that OT is in some way related to "positive" symptoms. Therefore, a role for increased central OT secretion in the pathophysiology of schizophrenic symptoms can be suggested. However, the fact that neuroleptic treatment, although clinically effective, does not induce a decrease in CSF OT concentrations argues against a direct role for this neuropeptide in the pathogenesis of schizophrenia. Moreover, increased hN_PII levels also has been described in women during pregnancy (Legros *et al.*, 1969) and following estrogen injection (Legros & Franchimont, 1970; Robinson *et al.*, 1973), and in men during acute and chronic alcoholism (Legros *et al.*, 1983), all conditions clearly not related to schizophrenic or schizophrenic symptoms. Future work will be necessary to delineate the pathophysiological meaning of an increased oxytocinergic and decreased vasopressinergic functions in schizophrenia.

Acknowledgements: This work was supported by a grant from the Fonds Facultaire de la Recherche Médicale, Faculty of Medicine, University of Liége.

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