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ORIGINAL INVESTIGATION

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Diazepam-like effects of a fish protein hydrolysate (Gabolysat PC60) on stress responsiveness of the rat pituitary-adrenal system and sympathoadrenal activity

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Abstract *Rationale*: Gabolysat PC60 is a fish protein hydrolysate with anxiolytic properties commonly used as a nutritional supplement. *Objective:* The diazepam-like effects of PC60 on stress responsiveness of the rat pituitary-adrenal system and on sympathoadrenal activity were studied. *Methods:* The activity of the pituitary-adrenal axis, measured by plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone (B) of the sympathoadrenal complex, measured by circulating levels of noradrenaline (NA) and adrenaline (A), and the gamma aminobutyric acid (GABA) content in the hippocampus and the hypothalamus were investigated in male rats which received daily, by an intragastric feeding tube, for 5 days running either diazepam (1 mg/kg) or PC60 (300 or 1200 mg/kg). Controls received only solvent (carboxymethylcellulose 1%). Six hours after the last force-feeding, the rats were subjected to 3 min ether inhalation or 30 min restraint and killed by decapitation 30 min after ether stress or at the end of restraint. *Results:* Baseline plasma levels of ACTH, B, NA and A were not affected by either diazepam or PC60. Both ether- and restraint-induced release of ACTH, but not B, were similarly and drastically reduced by diazepam and PC60 (1200 mg/kg). Both diazepam and PC60 (1200 mg/kg) deleted restraint-induced NA and A increases. Both treatments also reduced the ether-induced rise of A. Basal levels of GABA were significantly increased in both the hippocampus and the hypothalamus in PC60-treated rats and only in the hippocampus in diazepam-treated ones. In controls, ether inhalation as well as restraint increased GABA content of these two brain structures. In contrast, such stress procedures performed in PC60-treated rats reduced GABA content slightly in the hippocampus but significantly in the hypothalamus. In diazepam-treated rats, GABA content of the hypothalamus was unaffected by stresses but that of the hippocampus was slightly decreased. *Conclusions:* Present data suggest diazepam-like effects of PC60 on stress responsiveness of the rat pituitary adrenal axis and the sympathoadrenal activity as well as GABA content of the hippocampus and the hypothalamus under resting and stress conditions. These effects of PC60 agree with anxiolytic properties of this nutritional supplement, previously reported in both rats and humans.

Key words ACTH · Corticosterone · GABA · Noradrenaline · Adrenaline · Stress · Rat · Diazepam

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Introduction

Different types of stressors regarded by some investigators as "psychological" stressors (type restraint) or as "systemic" ones (type ether inhalation) alter brain concentrations and turnover of biogenic amines (review in Petty et al. 1996) and stimulate sympathoadrenal activity as well as the hypothalamic-pituitary-adrenocortical axis (Bernet et al. 1998). The neuroregulation of the corticostimulating function of the adult adenohypophysis includes, besides hypothalamic corticoliberin (CRH), stimulating neurotransmitters such as catecholamines, mainly noradrenaline (review in Pacak et al. 1995), and inhibitory ones such as galanin (Hooi et al. 1990) or GABA (gamma aminobutyric acid) (Keim and Shekhar 1996). The responsiveness to several types of stress was reported to be affected by exogenous drugs or possibly by diet. Indeed, a hydrolysate of fish proteins arising mainly from cod (Gadus) and mackerel (Scomber), commercially named PC60, and its derivative, called Stabilium 200, was reported to reduce anxiety in humans (Dorman et al. 1995) and to improve memory and learning performances in rats (Le Poncin 1996a) and patients (Le Poncin 1996b).

The aim of the present work was to investigate putative effects of PC60 given daily to rats for 5 days running as nutritional supplement on the responsiveness to two types of stressors (ether inhalation and restraint) and to compare PC60 to a potent anxiolytic drug, diazepam (Valium) which acts on benzodiazepine receptors. We used as reliable signs of the pituitary-adrenal activation, plasma levels of both adrenocorticotropic hormone (ACTH) and corticosterone (B) and as evidence of sympathoadrenal stimulation, plasma levels of noradrenaline (NA) and adrenaline (A). Moreover, we determined the GABA content of two brain structures, the hypothalamus and the hippocampus.

Materials and methods

Animals

Experiments were performed on male Wistar rats (250 g) purchased from IFFA-CREDO breeding (69 L'Arbresle, France) and transferred into individual cages, several days before being treated. They were housed in a light-controlled room (light period from 7 a.m. to 7 p.m.) with rat chow and tap water available ad libitum.

Animal use accreditation by the French Ministry of the Agriculture (no. 04860) has been delivered to our laboratory for experimentation with rats.

Treatment of rats

The rats received, by an intragastric feeding tube, every morning (between 8 a.m. and 9 a.m.), for 5 days running, either PC60 [300 or 1200 mg/kg scattered in 0.5 ml 1% (w:v) carboxymethyl cellulose (CM52, Whatman ; CMC 1%)] or diazepam (Valium, Roche Laboratories, 92 Neuilly, France; 1 mg/kg dissolved in 0.5 ml CMC). The composition of PC60 is reported in Table 1. Glutamic acid is one of the main amino acids present in PC60. Controls received exclusively 0.5 ml of vehicle (CMC 1%).

Six hours after the last force-feeding (i.e. between 2 p.m. and 3 p.m.), some animals of each experimental group were very rapidly killed by decapitation under resting conditions to collect their blood. while other rats were subjected to ether inhalation (3 min) or restraint (30 min) in a plastic box (65×200×60 mm), before being killed by decapitation at the end of the stress period (restraint) or 30 min later (ether inhalation).

The time between the removal of one rat from its cage or the restraint box and decapitation never exceeded 5 s.

Brain collection

Immediately after decapitation, the head was frozen in isopentane (–160°C) and following this rapid freezing (15 s), the head was stored in a refrigerator at –80°C until dissection of the brain structures (hypothalamus and hippocampus) to measure their GABA content.

Blood collection

Blood samples were collected in less than 10 s in polypropylene tubes containing EDTA 5%. After centrifugation, plasma samples were stored at –20°C for ACTH, B, NA and A determinations.

Assays

Plasma ACTH levels were determined by RIA according to the previously detailed procedure (Chatelain and Dupouy 1985). Fifty percent inhibition of ^{125}I-ACTH binding to the antibody was obtained with 25 pg of unlabelled ACTH. The variability was 3.22% (n=18) for intra-assay and 9.62% (n=15) for interassay.

Corticosterone levels in plasma samples were determined by radioimmunoassay according to the protocol previously described (Laborie et al. 1997).

Plasma catecholamine levels were determined by high-performance liquid chromatography (HPLC) and electrochemical detection as previously detailed (Bernet et al. 1991).

Table 1 Composition of Gabolysat PC60

Dry extract (g/kg)	>960	Molecular weight of the peptides	
Protein fraction (N×6.25) (g/kg)	800±20	≥1.8 kD	0%
N amino acid/N total (%)	320±20	≥0.6 and <1.8 kD	35±5%
Lipid fraction (g/kg)	80±20	≥0.3 and <0.6 kD	30±5%
Mineral fraction (g/kg)	≤90	<0.3 kD	35±5%
Amino acids (g/kg)			
Alanine	45	Lysine	43
Arginine	42	Methionine	15
Aspartic acid	44	Phenylalanine	23
Cystine	06.4	Serine	32
Glutamic acid	105	Taurine	28
Glycine	60	Threonine	26
Histidine	08	Tryptophane	04
Isoleucine	24	Valine	25
Leucine	40	Tyrosine	15
Vitamin E	40 mg/kg	Polyunsaturated fatty acids	
		Omega 3 family (of the lipid fraction)	>28%
Minerals (mg/kg)			
Calcium	300	Iron	5
Magnesium	3	Copper	3
Phosphorus	4	Zinc	2.5
Cobalt	1	Molybdenum	1

The hypothalamus and the hippocampus were sonicated in 50% methanol (5 µl/mg brain tissue) at 0°C. After centrifugation, the GABA content of the supernatant was determined by HPLC and electrochemical detection according to the procedure of Kehr and Ungerstedt (1988).

Statistical analysis

All measures were subjected to two-way analysis of variance (drug treatments×experimental conditions). To evaluate significant interactions the differences between two groups were determined by one-way analysis of variance and Dunnett's test was used for post hoc comparisons.

Differences were considered statistically significant if $P<0.05$.

All data are presented as means±SEM.

Results

Body growth

The rats treated with PC60 at the dose of 300 mg/kg per day for 5 days running showed body growth similar to that observed in controls given CMC 1% as vehicle (J1: 253.24±1.24 g; *n*=24, for controls ; 255.18±1.53 g, *n*=28, for PC60 treated rats ; $P>0.05$; J5: 283.12±1.72 g; *n*=24, for controls ; 284.28±1.66 g, *n*=28 for PC60-treated rats ; $P>0.05$). Rats given PC60 at the upper dose (1200 mg/kg per day) for 5 days running showed body weight increase similar to controls (data not shown).

Effects of PC60 and diazepam on the hormones of the pituitary-adrenal axis

At rest

PC60 at the two investigated doses (300 or 1200 mg/kg per day) or diazepam were unable significantly to affect ($P>0.05$) basal levels of ACTH (Fig. 1) or B (Fig. 2) on day 5 of treatment. Circulating levels of ACTH and B were similar to those observed in controls (rats given CMC 1%) ($P>0.05$).

Under stress conditions

In controls, ether-inhalation induced a drastic increase of both ACTH ($P<0.001$) (Fig. 1) and B levels ($P<0.001$) (Fig. 2).

Ether inhalation increased significantly circulating levels of ACTH ($P<0.001$) (Fig. 1) and B (Fig. 2) in rats given PC60 (300 or 1200 mg/kg/day) or diazepam. Nevertheless, plasma ACTH concentrations were slightly but not significantly weaker ($P>0.05$) in rats treated with the low dose of PC60 (300 mg/kg per day) exposed to ether inhalation when compared to controls. In contrast, ether-induced plasma ACTH increases were significantly weaker in rats treated with either the high dose of PC60 (1200 mg/kg per day) ($P<0.01$) or diazepam ($P<0.001$) (Fig. 1). Moreover, plasma ACTH levels were similar in

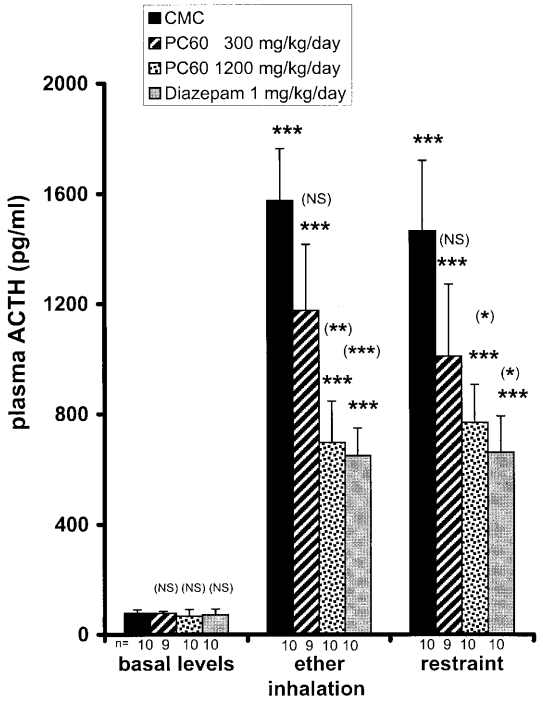

Fig. 1 Plasma ACTH levels before (basal levels) and under stress conditions (ether inhalation, restraint) in rats treated with CMC, PC60 or diazepam (*DZ*). *n*=number of rats used in each group. Statistical analysis: PC60 or DZ versus CMC: (NS) $P>0.05$, (*) $P<0.05$, (**) $P<0.01$, (***) $P<0.001$; CMC or PC60 or DZ under stress conditions versus corresponding basal levels: ***$P<0.001$

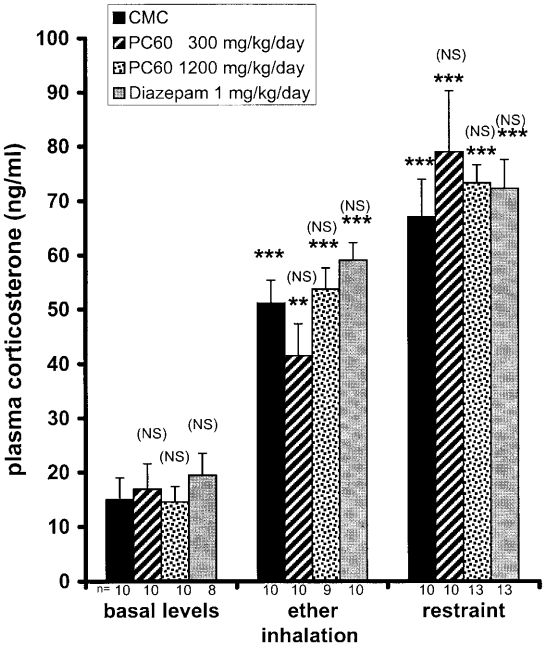

Fig. 2 Plasma corticosterone levels before (basal levels) and under stress conditions (ether inhalation, restraint) in rats treated with CMC, PC60 or diazepam (*DZ*). *n*=number of rats used in each group. Statistical analysis: PC60 or DZ versus CMC: (NS) $P>0.05$; CMC or PC60 or DZ under stress conditions versus corresponding basal levels: **$P<0.01$, ***$P<0.001$

these two latter experimental groups (P>0.05). In spite of such differences for circulating ACTH between controls and PC60- or diazepam-treated rats subjected to ether inhalation, plasma levels of B were not significantly different (P>0.05) in stressed animals of the four experimental groups (Fig. 2).

In controls, 30-min restraint increased both circulating levels of ACTH (P<0.001) (Fig. 1) and B (P<0.001) (Fig. 2).

The rise in plasma ACTH levels induced by restraint was significantly and similarly reduced (P<0.05) in rats treated with either PC60 (1200 mg/kg per day) or diazepam (Fig. 1). ACTH response to restraint was slightly but not significantly (P>0.05) reduced by treatment with PC60 at the lowest dose (300 mg/kg per day).

In contrast, PC60 (300 and 1200 mg/kg/day) or diazepam were unable to affect significantly plasma corticosterone elevation induced by restraint (Fig. 2).

Effects of PC60 and diazepam on circulating catecholamines (NA and A)

At rest

PC60 at the two investigated doses (300 or 1200 mg/kg/day) as well as diazepam did not significantly modify (P>0.05) plasma levels of NA (Fig. 3) or A (Fig. 4) which were similar to those found in controls (rats given CMC 1%).

Under stress conditions

Ether inhalation did not significantly affect (P>0.05) circulating levels of NA in either controls or in rats treated with PC60 at the two doses or diazepam (Fig. 3). In contrast, circulating levels of A were significantly increased by ether-inhalation in controls (P<0.01) and to a lesser extent in rats treated with PC60 (1200 mg/kg per day) (P<0.05) (Fig. 4). However, ether-induced elevation of A levels was lacking in rats treated with either PC60 (300 mg/kg per day) or diazepam (Fig. 4).

In controls, 30-min restraint increased significantly plasma concentrations of both NA (P<0.01) (Fig. 3) and A (P<0.01) (Fig. 4). In rats treated with PC60 (300 mg/kg per day) stress-induced rises in plasma NA (P<0.01) and A (P<0.05) were similar to those observed in controls (P>0.05) (Fig. 3 and Fig. 4). In contrast, the treatment of rats with either PC60 (1200 mg/kg per day) or diazepam deleted restraint-induced elevation of both NA and A in the circulation (Fig. 3 and Fig. 4). Moreover, plasma levels of A in diazepam-treated and stressed rats were significantly weaker that basal levels (diazepam-treated and unstressed rats) (P<0.01) (Fig. 4).

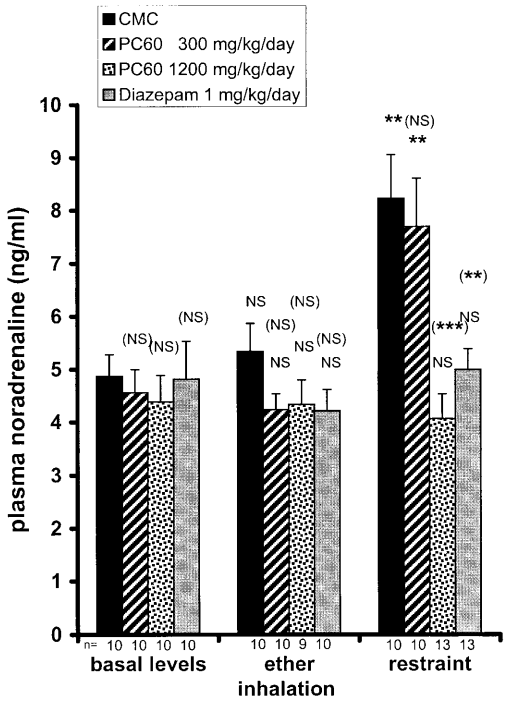

Fig. 3 Plasma noradrenaline levels before (basal levels) and under stress conditions (ether inhalation, restraint) in rats treated with CMC, PC60 or diazepam (*DZ*). *n*=number of rats used in each group. Statistical analysis: PC60 or DZ versus CMC: (NS) P>0.05, (**) P<0.01, (***) P<0.001; CMC or PC60 or DZ under stress conditions versus corresponding basal levels: NS P>0.05, **P<0.01

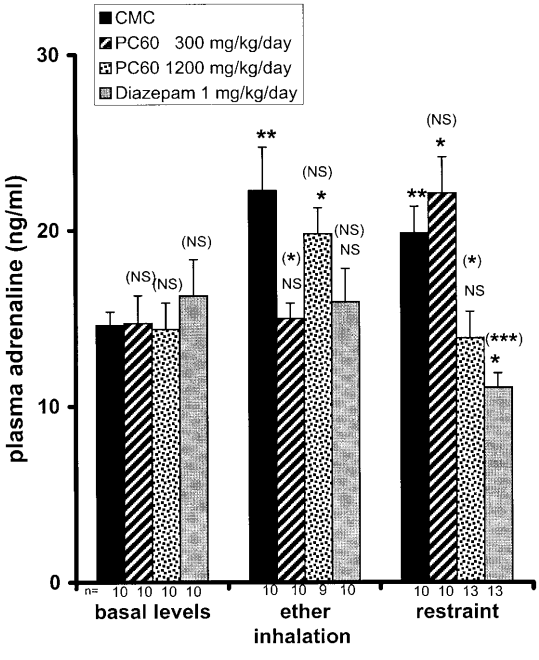

Fig. 4 Plasma adrenaline levels before (basal levels) and under stress conditions (ether inhalation, restraint) in rats treated with CMC, PC60 or diazepam (*DZ*). *n*=number of rats used in each group. Statistical analysis: PC60 or DZ versus CMC: (NS) P>0.05, (*) P<0.05, (***) P<0.001; CMC or PC60 or DZ under stress conditions versus corresponding basal levels: NS P>0.05, *P<0.05, **P<0.01

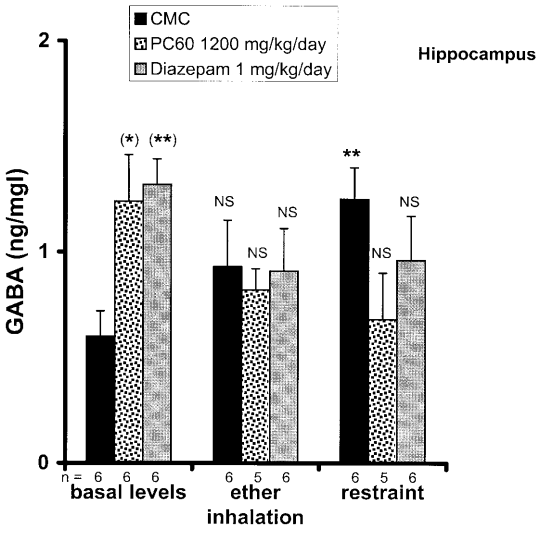

Fig. 5 GABA content in the hippocampus before (basal levels) and under stress conditions (ether inhalation, restraint) in rats treated with CMC, PC60 or diazepam (*DZ*). *n*=number of rats used in each group. Statistical analysis: PC60 or DZ versus CMC: (*) $P<0.05$, (**) $P<0.01$; CMC or PC60 or DZ under stress conditions versus corresponding basal levels: NS $P>0.05$, **$P<0.01$

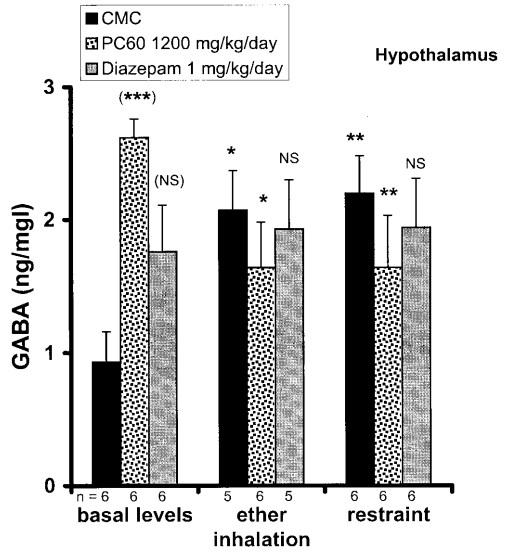

Fig. 6 GABA content in the hypothalamus before (basal levels) and under stress conditions (ether inhalation, restraint) in rats treated with CMC, PC60 or diazepam (*DZ*). *n*=number of rats used in each group. Statistical analysis: PC60 or DZ versus CMC: (NS) $P>0.05$, (***) $P<0.001$; CMC or PC60 or DZ under stress conditions versus corresponding basal levels: NS $P>0.05$, *$P<0.05$, **$P<0.01$

Effects of PC60 and diazepam on GABA content of the hippocampus and the hypothalamus

Basal levels of GABA were significantly increased in both the hippocampus ($P<0.05$) (Fig. 5) and the hypothalamus ($P<0.001$) (Fig. 6) in PC60 (1200 mg/kg per day) treated rats. diazepam also increased basal levels of GABA in these two structures of the brain but the rise was significant only in the hippocampus ($P<0.01$) (Fig. 5 and Fig. 6).

In controls (CMC-treated rats), ether inhalation induced a slight but not significant ($P>0.05$) elevation of GABA content in the hippocampus (Fig. 5) but a significant one in the hypothalamus ($P<0.05$) (Fig. 6). Restraint induced significant increase ($P<0.01$) in both the hippocampus (Fig. 5) and the hypothalamus (Fig. 6). In contrast, PC60-treated rats showed, in response to ether inhalation or restraint, a slight but not significant decrease of GABA concentration in the hippocampus (Fig. 5) but a significant decrease in the hypothalamus (Fig. 6). In diazepam-treated rats, GABA content of the hippocampus was slightly but not significantly decreased by the two stress procedures (Fig. 5); however, the GABA content of the hypothalamus was not significantly affected.

Discussion

The treatment of rats, once a day, with PC60 (300 and 1200 mg/kg) for 5 consecutive days did not affect body weight increase and, under resting conditions, plasma levels of the two main hormones of the pituitary-adrenal axis (ACTH and B) and of catecholamines (NA, A). Similarly, diazepam at the dose of 1 mg/kg did not significantly affect , 6 h after the last injection, basal levels of these blood parameters. However, diazepam administered i.p. at higher doses (from 1.5 to 6.0 mg/kg) 1 h prior to restraint increased non-stress baseline plasma B levels in a dose-dependent fashion (Kalman et al. 1997). In a preliminary experiment (data not reported), we studied the evolution in course of time of ACTH, B and A in rats treated for 5 days running with either PC60 (300 mg/kg per day) or vehicle (CMC 1%) and thereafter subjected to 3 min ether inhalation, according to the present procedure. In these two experimental groups, ether exposure induced drastic elevation of these blood parameters that was highest 30 min later. We previously reported that30 min after the end of 3 min ether inhalation as well as at the end of a 30-min restraint period, ACTH, B and catecholamines were significantly increased in the plasma of male rats (Bernet et al. 1998). Moreover, NA found in the plasma before and during ether or restraint stress could arise from both adrenal medulla and noradrenergic nerve endings, while A arises mainly from the adrenergic chromaffin cells of the adrenals (Bernet et al. 1998). As ether inhalation did not increase basal levels of NA, whereas there was a slight increase in the plasma A in contrast to restraint, one can speculate that the former stress was more moderate and shorter that the latter to stimulate noradrenergic nerve endings.

Adrenal medulla was mainly controlled by splanchnic nerve via acetylcholine release and nicotinic receptors activation (Laborie et al. 1997). These data support the idea that the killing of the rats 30 min after ether inhalation or at the end of a 30-min restraint period was an appropriate time to investigate responsiveness of both the pituitary adrenal and the sympathoadrenal activities.

Present data also suggest that PC60 at the lowest tested dose (300 mg/kg) was able to suppress ether-induced increase of circulating A that could indicate activation of the sympathoadrenal complex. Moreover, PC60 mimics diazepam effects on catecholamine responsiveness to the ether-stress. The effects of benzodiazepines on the hypothalamo-pituitary-adrenal (HPA) axis are far from being clear. Indeed, conflicting results have been shown by many authors. Concerning the HPA axis, present data suggest that PC60 at the highest tested dose (1200 mg/kg) as well as diazepam significantly reduced ACTH release under stress conditions such as ether inhalation or restraint. This observed decrease of ACTH release cannot be attributable to habituation after 5 days of handling and forcible feeding because the control rats similarly handled and fed always show a drastic activation of the HPA under ether-inhalation or restraint stress. Furthermore, chronic repetitive stress has been suggested to cause a shift in ACTH secretagogues towards arginine vasopressin (Ma and Lightman 1998), but our investigation does not allow us to confirm this suggestion, as we did not assay CRF and AVP. Diazepam, administered i.p. at a higher dose than that used in our study, attenuated the stress-induced increase in corticosterone following 5 days of diazepam+restraint treatment (Kalman et al. 1997). However, B plasma levels were not significantly affected in spite of reduced ACTH secretion. To explain the apparent discrepancy between plasma ACTH values and the glucocorticoid values, one can speculate that ACTH levels observed in PC60- or diazepam-treated rats subjected to stress (ether inhalation or restraint) were nevertheless sufficient to stimulate the adrenal cortex fully.

The highest dose of PC60 (1200 mg/kg) as well as diazepam (1 mg/kg) deleted restraint-induced activation of the sympathoadrenal complex. According to our data, PC60 showed diazepam-like effects on the responsiveness of both pituitary-adrenal axis and sympathoadrenal complex to two types of stressors, one mainly systemic (ether inhalation) and one mainly psychological (restraint).

The mechanisms by which PC60 presents anti-stress properties are nowadays unknown. Nevertheless, as PC60 contains a high level of glutamic acid, a GABA precursor, in comparison with other amino acids, PC60 treatment could affect GABA content of some brain structures as observed in both the hippocampus and the hypothalamus. In contrast the effects of diazepam on the central nervous system are very documented. Diazepam is a benzodiazepine derivative drug that is a potent anxiolytic by acting on benzodiazepine receptors which are concentrated in several regions of the brain, especially cortex, cerebellum and limbic structures such as the hippocampus and amygdala (Schoch et al. 1985). The benzodiazepine receptor is a part of a macromolecular complex with the GABA receptor which has been cloned (Schofield et al. 1987). Benzodiazepines act by facilitating inhibitory GABA transmission in the central nervous system (Haefely 1985). However, GABA release was not changed in hippocampal slices from rats treated 5 days with diazepam (4 mg/kg i.p.) but was significantly increased after 21 days of treatment (Hitchcott et al. 1990). Moreover, rats treated with diazepam for 5 days (present data) showed an increase of GABA content in the hippocampus and less extensively in the hypothalamus. Then, concerning basal levels of GABA in these two brain structures (hippocampus, hypothalamus), PC60 showed diazepam-like effects.

As GABA concentration in brain structures depends of both synthesis and release, reduction of GABA content could reflect in part increased secretion.

Stress-induced decreased of GABA content, which was higher in the hypothalamus than in the hippocampus of PC60-treated rats could suggest increased GABA release. A similar but weaker effect of the two stresses on the GABA content of the hippocampus was also observed in diazepam-treated rats. Present data in rats under stress conditions suggest once again that PC60 shows some diazepam-like effects.

GABA is one of the principal inhibitors of ACTH release (Jones and Gillham 1988). GABA-ergic neurons have been found to synapse on corticoliberin (CRH)- and vasopressin (AVP)-containing cell bodies (Meister et al. 1988; Decavel et al. 1989). GABA inhibited CRH release from medial basal hypothalamic fragments in vitro (Calogero et al. 1988) and CRH neurons in the paraventricular nucleus (PVN) have inhibitory GABAergic inputs which regulate CRH release (Tasker and Dudek 1993). $GABA_A$ receptor blockade in the dorsomedial hypothalamic nucleus of male rats increases heart rate, blood pressure and plasma levels of both ACTH and B (Keim and Shekhar 1996). A decrease of the plasma ACTH and B concentrations was reported in female rats treated with diazepam administered i.p.; this fall of ACTH and B levels was reversed by an antagonist of central, but not peripheral, benzodiazepine receptors (Privac and Pericic 1993). These GABA receptor-mediated central effects of diazepam and putative increase of GABA release could explain why our rats treated with this drug for 5 consecutive days showed reduced responsiveness of the pituitary corticostimulating function (ACTH) under stress conditions. Similarly, in PC60-treated rats, putative increase of GABA release could also explain reduced stress-activation of the ACTH secretion.

On the other hand, PVN receives projections from ascending catecholaminergic pathways and catecholamines have an important role in the activation of CRH neurons during stress (reviews in Aguilera 1994; Paulmyer-Lacroix et al. 1995).

As diazepam was reported to attenuate psychological stress-induced increases in NA release in rat brain regions (Tanaka et al. 1991; Nakane et al. 1994) one can explain deleterious actions of this drug on stress responsiveness. PC60 has been reported to exert diazepam-like effects on stress responses and, moreover, that a nutritional supplement called “Stabilium”, derived from PC60, reduced anxiety in college students (Dorman et al.

1995). PC60 was reported to have overall positive effects on astheno-depressive patients (Bugard, Crocq and Barre, personal communication). The current data indicate that this fish protein hydrolysate, as well as diazepam, alters brain content of GABA in both the hippocampus and the hypothalamus. However, according to our observations, it is not clear whether the reduced activation of the HPA axis by stress reflects the anxiolytic effect of the PC60 or results from a direct effect on the central control of this axis.

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References

Aguilera G (1994) Regulation of pituitary ACTH secretion during chronic stress. Front Neuroendocrinol 15:321–350

Bernet F, Maubert E, Deloof S, Bernard J, Montel V, Dupouy JP (1991) Effects of estrous cycle and of in vivo unilateral or bilateral adrenal demedullation on the distribution of norepinephrine and epinephrine between different zones of the adrenal. J Neuroendocrinol 3:523–528

Bernet F, Dedieu JF, Laborie C, Montel V, Dupouy JP (1998) Circulating neuropeptide Y (NPY) and catecholamines in rat under resting and stress conditions. Arguments for extra-adrenal origin of NPY, adrenal and extra-adrenal sources of catecholamines. Neurosci Lett 250:45–48

Calogero AE, Gallucci WT, Chrousos GP, Gold PW (1988) Interaction between GABAergic neurotransmission and rat hypothalamic corticotropin-releasing hormone secretion in vitro. Brain Res 463:28–36

Chatelain A, Dupouy JP (1985) Adrenocorticotrophic hormone in the anterior and neurointermediate lobes of the rat during the perinatal period: polymorphism, biological and immunological activities of ACTH. Biol Neonate 47:235–248

Decavel C, Dubourg P, Leon-Henri B, Geffard M, Calas A (1989) Simultaneous immunogold labeling of GABAergic terminals and vasopressin-containing neurons in the rat paraventricular nucleus. Cell Tissue Res 255:77–80

Dorman T, Bernard L, Glaze P, Hogan J, Skinner R, Nelson D, Bowker L, Head D (1995) The effectiveness of Garum armoricum (stabilium) in reducing anxiety in college students. J Adv Med 8:193–200

Haefely W (1985) The biological basis of benzodiazepine action. In: Smith DE, Wesson DR (eds) The benzodiazepines. MTP Press, Lancaster, pp 7–41

Hitchcott PK, File SE, Ekwuru M, Neal MJ (1990) Chronic diazepam treatment in rats causes long-lasting changes in central [^{3}H]5-hydroxytryptamine and [^{14}C]γ-aminobutyric acid release. Br J Pharmacol 99:11–12

Hooi SC, Maiter DM, Martin JB, Koenig JI Galaninergic mechanisms are involved in the regulation of corticotropin and thyrotropin secretion in the rat. Endocrinology 127:2281–2289

Jones MT, Gillham B (1988) Factors involved in the regulation of adrenocorticotropic hormone/beta-lipotropic hormone. Physiol Rev 68:743–818

Kalman BA, Kim PJ, Cole MA, Chi MS, Spencer RL (1997) Diazepam attenuation of restraint stress-induced corticosterone levels is enhanced by prior exposure to repeated restraint. Psychoneuroendocrinology 22:349–360

Kehr J, Ungersted U (1988) Fast HPLC estimation of γ-aminobutyric acid in microdialysis perfusates: effect of nipecotic and 3-mercaptopropionic acids. J Neurochem 51:1308–1310

Keim SR, Shekhar A (1996) The effects of $GABA_A$ receptor blockade in the dorsomedial hypothalamic nucleus on corticotrophin (ACTH) and corticosterone secretion in male rats. Brain Res 739:46–51

Laborie C, Bernet F, Dutriez-Casteloot I, Lesage J, Dupouy JP (1997) Effect of cholinergic blockade on glucocorticoid regulation of NPY and catecholamines in the rat adrenal gland. Neuroendocrinology 66:98–105

Le Poncin M (1996a) Experimental study: stress and memory. Eur Neuropsychopharmacol 6:110-P10-2

Le Poncin M (1996b) Nutrient presentation of cognitive and memory performances. Eur Neuropsychopharmacol 6:187-P19-4

Ma XM, Lightman S (1998) The arginine vasopressin and corticotrophin-releasing hormone gene transcription responses to varied frequences of repeated stress in rats. J Physiol (Lond) 510:605–614

Meister B, Hokfelt T, Geffard M, Oertel W (1988) Glutamic acid decarboxylase- and gamma-aminobutyric acid-like immunoreactivities in corticotropin-releasing factor-containing parvocellular neurons of the hypothalamic paraventricular nucleus. Neuroendocrinology 48:516–526

Nakane H, Shimizu N, Hori T (1994) Stress-induced norepinephrine release in the rat prefrontal cortex measured by microdialysis. Am J Physiol 267:R1559–1566

Pacak K, Palkovits M, Kopin IJ, Goldstein DS (1995) Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympathoadrenal activity: in vivo microdialysis studies. Front Neuroendocrinol 16:89–150

Paulmyer-Lacroix O, Guillaume V, Anglade G, Grino M, Oliver C (1995) Regulation de la fonction corticotrope dans les situations de stress. Ann d'Endocrinol 56:245–251

Petty F, Kramer GL, Larrison AL (1996) Neurochemistry of stress: regional brain levels of biogenic amines and metabolites with ten different stressors. Biogen Amines 12:377–394

Privac N, Pericic D (1993) Inhibitory effect of diazepam on the activity of the hypothalamic pituitary-adrenal axis in female rats. J Neural Transm Gen Sect 92:173–186

Schoch P, Richards JG, Haring P, Takacs B, Stahli C, Staehelin T, Haefely W, Mohler H (1985) Co-localization of GABA receptors and benzodiazepine receptors in the brain shown by monoclonal antibodies. Nature 314:168–171

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA, Seeburg PH, Barnard EA (1987) Sequence and functional expression of the GABA A receptor shows a ligand-gated receptor super family. Nature 328:221–227

Tanaka M, Tsuda A, Yokoo H, Yoshida M, Mizoguchi K, Shimizu T (1991) Psychological stress-induced increases in noradrenaline release in rat brain regions are attenuated by diazepam, but not by morphine. Pharmacol Biochem Behav 39:191–195

Tasker J, Dudek FE (1993) Local inhibitory synaptic inputs to neurones of the paraventricular nucleus in slices of rat hypothalamic. J Physiol 469:179–192