REVIEW ARTICLE

Benzodiazepines and anterior pituitary function

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ABSTRACT. Benzodiazepines (BDZ) are one of the most prescribed classes of drugs because of their marked anxiolytic, anticonvulsant, muscle relaxant and hypnotic effects. The pharmacological actions of BDZ depend on the activation of 2 specific receptors. The central BDZ receptor, present in several areas of the central nervous system (CNS), is a component of the GABA-A receptor, the activation of which increases GABAergic neurotransmission and is followed by remarkable neuroendocrine effects. The peripheral benzodiazepine receptors (PBR), structurally and functionally different from the GABA-A receptor, have been shown in peripheral tissues but also in the CNS, in both neurones and glial cells, and in the pituitary gland. BDZ receptors bind to a family of natural peptides called endozepines, firstly isolated from neurons and glial cells in the brain and then in several peripheral tissues as well. Endozepines modulate several central and peripheral biological activities, including some neuroendocrine functions and synthetic BDZ are likely to mimic them, at least partially. BZD, especially alprazolam (AL), possess a clear inhibitory influence on the activity of the HPA axis in both animals and humans. This effect seems to be mediated at the hypothalamic and/or suprahypothalamic level *via* suppression of CRH. The strong negative influence of AL on hypothalamic-pituitary-adrenal (HPA) axis agrees with its peculiar efficacy in the treatment of panic disorders and depression. BZD have also been shown to increase GH secretion via mechanisms mediated at the hypothalamic or supra-hypothalamic level, though a pituitary action cannot be ruled out. Besides the impact on HPA and somatotrope function, BDZ also significantly affect the secretion of other pituitary hormones, such as gonadotropins and PRL, probably acting through GABAergic mediation in the hypothalamus and/or in the pituitary gland. In all, BDZ are likely to represent a useful tool to investigate GABAergic activity and clarify its role in the neuroendocrine control of anterior pituitary function; their usefulness probably overrides what had been supposed before.

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INTRODUCTION

Benzodiazepines (BDZ) are one of the most prescribed classes of drugs because of their marked anxiolytic, anticonvulsant, muscle relaxant and hypnotic effects (1). It has been shown that many of the pharmacological actions of BDZ are mediated by specific binding sites, distributed not only in the central nervous system (CNS) but also in peripheral tissues. In the CNS BDZ bind a specific BDZ binding site which is a component of the GABA-A receptor (1-3). This receptor activation facilitates brain GABAergic neurotransmission (4, 5), which plays the most important inhibitory influence in the CNS (1). Moreover, GABA has been found to modulate pituitary function by acting at the hypothalamic level where influences hypophysiotropic neurohormone release, and/or directly on the pituitary gland, where different GABA receptor subtypes have been detected (6-13).

There is evidence that clinical actions of BDZ are mainly mediated by inhibitory influence on 3 classical stress systems: sympatoneural and adrenomedullary systems and hypothalamic-pituitary-adrenal (HPA) axis. BDZ-induced GABAergic activation counteracts HPA hyperactivation associated to stressful conditions and there is evidence that CRH *per se* increases vigilance, attention, anxiogenic behavior and reduces feeding and hypothalamic-pituitary-gonadal activity (1, 14-17). Moreover, alterations in the balance of central activating and inhibiting

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neurotransmission might be involved in the pathophysiology of some psychiatric disorders, such as major depression or panic disorders (18-20). Among BDZ alprazolam (AL), a triazolobenzodiazepine with peculiar efficacy in the treatment of panic disorders and depression (21-23), possesses the most remarkable inhibitory influence on ACTH and F release (see below for references).
Besides the impact on HPA axis, BDZ also significantly affect the secretion of other pituitary hormones, particularly GH, gonadotropins and PRL, probably acting through GABAergic mediation in the hypothalamus and/or in the pituitary gland (see below for references).
On the other hand, a different class of BDZ receptor named "peripheral benzodiazepine receptors" (PBR) has been shown in peripheral tissues but also in the CNS, in both neurones and glial cells (24, 25). The activation of these receptors plays a critical role in steroidogenetic processes leading to the synthesis of both neurosteroids and steroid hormones in peripheral glands (24, 25). PBR activation might play also some modulatory influence on the activity of the anterior pituitary gland (24, 25). It has to be emphasized that BDZ receptors including PBR bind a family of natural peptides called "endozepines". An 86-amino-acid polypeptide, named diazepam-binding inhibitor (DBI), was the first "endozepine" isolated from neurons and glial cells in the rat brain and then detected in several peripheral tissues too (24, 25). This and other related endozepines characterized thereafter modulate several central and peripheral biological activities, including some neuroendocrine functions (24-26). Thus, the neuroendocrine effects of BDZ are likely to reflect neuroendocrine actions of natural endozepines.
Based on the foregoing, this paper aims to review the present knowledge regarding the effects of BDZ on anterior pituitary function, with particular focus on data so far available in humans, in both physiological and physiopathological conditions.

FUNDAMENTALS IN BDZ

The pharmacological actions of BDZ depend on activation of their specific receptors. Two types of specific BDZ binding sites have so far been discovered: central and peripheral receptors (1, 24). High-affinity, saturable and stereospecific central binding sites for BDZ have been demonstrated in the cerebral cortex, cerebellum, hippocampus, amigdala, *locus coeruleus* and medial hypothalamus, but also at pituitary level, both in the anterior and posterior lobe (1, 27).
The central BDZ receptor is a component of the GABA-A receptor, a macromolecular complex with separate binding sites for GABA, BDZ and barbiturate-like substances (1-3). Activation of GABA-A-binding sites induces the opening of the chloride ionophore linked to GABA-A receptors, leading to membrane hyperpolarization and to inhibition of cell firing (28). There is evidence that BDZ binding to GABA-A receptor complex enhances GABA binding to its receptor site, thus increasing the activity of the GABAergic system (4, 5), which is the major inhibitory system in the brain. GABA, GABA-mimetic compounds and barbiturates increase the affinity of BDZ to their receptors, suggesting strict anatomical and functional link between BDZ and GABAergic pathways (1).
Three classes of ligands have been demonstrated to bind central BDZ receptor sites: agonists, which show anxiolytic and anticonvulsant effects, antagonists, which have no intrinsic pharmacological activity but block the actions of BDZ agonists, and inverse agonists, which show an intrinsic proconvulsant effect in animals (1, 29-31).
The actions of GABA/BDZ on the anterior pituitary function are believed to be mainly mediated at the hypothalamic level, where a great amount of neural connections between GABAergic pathways and neurons releasing hypophysiotropic neurohormones have been demonstrated (6-8). On the other hand, GABA is released in the hypothalamo-hypophyseal portal circulation from neurons located in the median eminence and, thus, can directly act on pituitary cells through specific GABA receptors, including GABA-A subtypes (11, 32-35). Thus, this evidence indicates the existence of a direct pituitary action of BDZ to modulate anterior pituitary function.
The peripheral type of BDZ receptor is a different class of binding sites for BDZ, located on the outer mitochondrial membrane, not functionally linked to the GABA regulated chloride channel and showing different selectivity to ligands (24, 25). PBR are present in high concentrations in steroid-producing tissues, such as adrenal cortex, testis, ovary, in the kidney and also in brain glial cells, hypothalamus and pituitary (24, 25, 36). In both peripheral tissues and brain these receptors play a crucial role in the regulation of steroidogenesis: they control the first step of the steroid biosynthetic pathway, *i.e.* the cholesterol delivery from the outer to the inner mitochondrial membrane (24, 25).
PBR were found as the first target of natural ligands called endozepines. The endozepine family is derived from a brain polipeptide, named DBI, which was discovered as the first endogenous ligand for PBR though it also binds to the GABA/BDZ receptor complex (1, 24-26). DBI was isolated from rat brain

and, later, from bovine and human brain, in both neurones and glial cell, where PBR as well as classical BDZ receptors are also present (25). The primary structure of DBI has been shown to be well conserved during evolution, suggesting that this peptide plays important biological functions (37). DBI and other endozepines have also been found in several peripheral tissues, particularly in the adrenal cortex, testis and ovary, where they are deeply involved in the control of steroidogenesis (25). The activation of PBR by endozepines in glial cells induces the synthesis of neurosteroids, which, in turn, modulate neuronal activity and brain function through the modulation of GABA/BDZ receptors which, as anticipated, are bound by endozepines (25).
The evidence that GABAergic pathways are present in high density in the hypothalamus and median eminence (34, 35) coupled with the demonstration of both GABA/BDZ and PBR in the pituitary gland (11, 32-35, 38, 39) suggested a role for endozepines as well as for BDZ in the modulation of the anterior pituitary function.
Besides the regulation of brain and peripheral steroid synthesis, it has been demonstrated that endozepines exert other remarkable activities, such as modulation of melanotropin release from pituitary cells, inhibition of insulin secretion from the pancreas, stimulation of intestinal cholecystokinin and regulation of cellular growth and differentiation (37).

BDZ AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

It has been clearly demonstrated that BDZ administration affects the activity of the HPA axis.
The first studies, generally obtained studying the effects of diazepam, reported conflicting results in both animals and humans. An inhibitory effect of diazepam on ACTH, corticosterone/F release was reported by some (40-43) but not by other Authors (44) in animal studies. Significant inhibitory effect of diazepam on HPA was found by some Authors in humans too (45-48), while others did not show any significant effect (49, 50). Moreover, other BDZ, such as oxazepam, triazolam or flurazepam, showed negligible or no effect on corticotrope and adrenal function in normal subjects (51, 52).
Clear evidence of the inhibitory effect of BDZ on HPA axis came from the studies using AL, a triazolobenzodiazepine which mainly acts as an agonist of the GABA/BDZ receptor complex (53-55). AL possesses the most remarkable inhibitory effect on HPA axis and this peculiar activity seems to explain its clinically efficacy not only as anxiolitic agent but also in the treatment of panic disorders and depressive disorders, in which a central HPA hyperactivation has been demonstrated (20-23).
AL shows a very high affinity for central BDZ receptors and a short elimination half-life (10-15 h), associated with small amounts of active metabolites, which are rapidly excreted in the urine (55-57). AL has been shown to significantly reduce basal corticotrope and adrenal secretion as well as the ACTH response to insulin-induced hypoglicemia in animals (58-60). Moreover, *in vitro* studies showed that basal and serotonin-stimulated CRH secretion was inhibited by AL in *locus coeruleus* and hypothalamus, with a potency 40 times higher than diazepam (58, 60).
In agreement with data in animals, in human studies AL has been demonstrated to significantly decrease urinary free F as well as circulating ACTH/F levels, enhancing the spontaneous decline of HPA activity in the morning hours, indicating a clear influence of BDZ on the basal HPA activity (20, 61). On the other hand, AL has been shown to significantly blunt the ACTH and/or F response to several stimulations, such as metabolic, mental stress, naloxone and AVP (62-67), while it totally abolished the ACTH response to hexarelin, a synthetic growth-hormone-releasing-peptide (GHRP) with ACTH-releasing effect (68). Moreover, AL did show a marked inhibitory effect even on ACTH rise induced by metyrapone or insulin-induced hypoglycemia, the most potent stimulations of corticotrope secretion (61, 69), clearly indicating a primary role of BDZ in the modulation of corticotrope function. Evidence that AL possesses a more marked inhibitory effect on HPA axis than other BDZ indicates the peculiar activity of this BDZ possibly reflecting its higher affinity for GABA/BDZ binding sites (70).
There is clear evidence that BDZ act through the modulation of GABAergic neurotransmission in both animals and humans (see previous pages for references) and that GABAergic pathways are involved in the neural control of HPA axis. In fact, AL as well as diazepam, although with a lesser potency, are able to suppress CRH secretion in the hypothalamus and *locus coeruleus* while neither AL nor GABA modified basal or CRH-stimulated ACTH release from pituitary cells (58, 60). Thus, the inhibitory action of BDZ/GABA on HPA axis mainly takes place at hypothalamic and/or suprahypothalamic level *via* suppression of CRH. Accordingly, recent studies showed that GABA and GABA-A agonists negatively influence CRH gene expression in the rat hypothalamus and that this effect is reversed by antagonists of GABA/BDZ binding sites (9, 71). On the other hand, other data indicated an AVP-mediated mechanism of GABA/BDZ on HPA axis. In

fact, GABA is synthesized in both supraoptic and paraventricular nuclei, where AVP is produced (72); moreover, GABAergic compounds reduce circulating AVP levels and inhibit AVP release from the hypothalamus and posterior pituitary in rats (73-75).
Evidence in humans that AL is able to inhibit the ACTH and F response to CRH-mediated stimuli, such as naloxone or metyrapone, to AVP but not to CRH (61-67, 76) (Fig. 1) reinforces the hypothesis that BDZ are likely to act within the CNS *via* inhibition of CRH release. However, other studies indicate that BDZ could act *via* AVP-mediated mechanisms (76-78), although some Authors did not find any significant effect of AL on AVP release in humans (63).
It has also been proposed that BDZ and GABA may influence HPA axis by acting at suprahypothalamic level. AL pre-treatment significantly decreases hippocampal CRH receptors in rats (79). Moreover, in humans, it abolishes the nocturnal ACTH and F rise induced by potassium canrenoate, an antagonist of both peripheral and hippocampal mineralocorticoid receptors (MR) (80). As the hippocampal MR activation is deeply involved in the glucocorticoid-mediated negative feed back mechanism (81), these results indicated the involvement of BDZ in the modulation of the negative feed-back mechanism induced by glucocorticoids. This is in agreement with the ability of AL to inhibit the stimulatory effect of the metyrapone-induced removal of corticosteroid feed back on corticotrope secretion (61).
Several studies showed that high BDZ doses reduce baseline and stress-induced NE turnover (82, 83). Moreover, BDZ have been found to inhibit cell firing and noradrenergic activity in the *locus coeruleus*, which provides central noradrenergic inputs to the CRH neurons, while GABA/BDZ receptors have been demonstrated on presynaptic axons and nerve terminals of noradrenergic neurons located in cerebral cortex and hippocampus (84-86). Thus, it has been hypothesized that BDZ may indirectly influence HPA activity *via* modulation of noradrenergic function. In agreement with this hypothesis, in humans the administration BDZ, namely AL, is followed by decrease in NE and/or NE metabolite levels (62, 63, 87). Moreover, AL antagonizes the behavioral and neuroendocrine effects of yohimbine and viceversa suggesting that the BDZ action on noradrenergic function could be mediated by the α2-adrenergic modulation (87).
Finally, although BDZ failed to modify CRH-induced ACTH secretion from rat pituitary (58), the existence of GABA/BDZ receptors in the pituitary gland (88) suggested a direct modulatory role of GABA and/or BDZ on corticotrope secretion induced by ACTH secretagogues other than CRH.

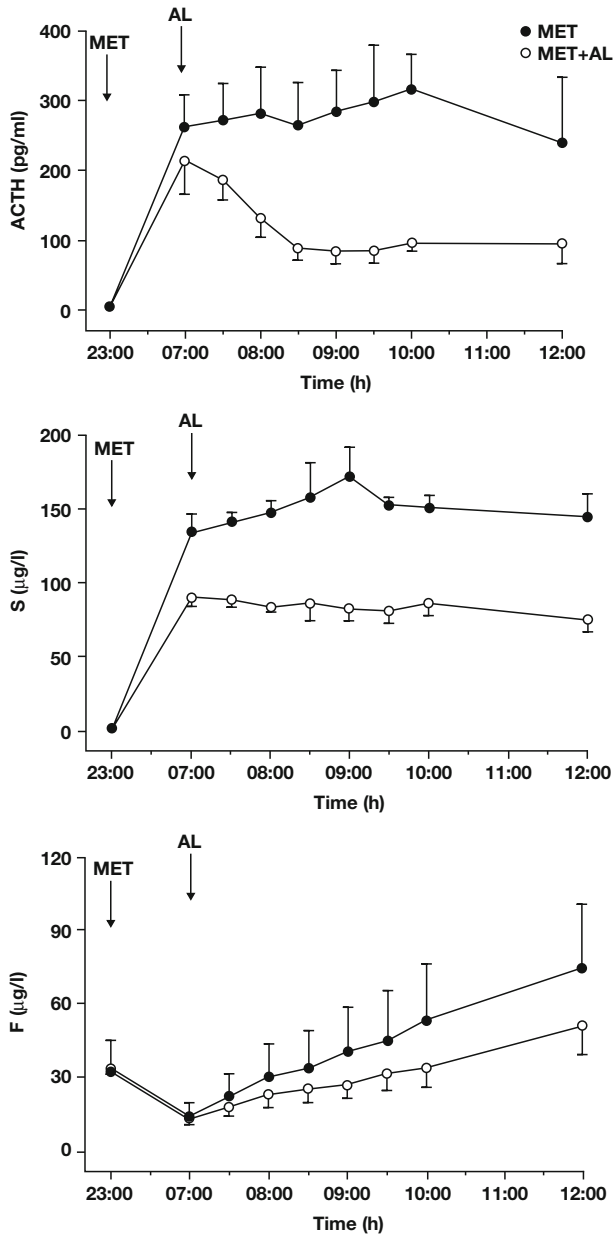

Fig. 1 - *Mean (±SE) ACTH, 11-desoxycortisol (S) and F levels after metyrapone (MET, 0.04 g/kg, orally, at 23:00 h the night before) alone and combined with alprazolam (AL, 0.02 mg/kg, orally, at 07:00 h) in normal subjects.*

As far as the influence of PBR on the activity of HPA axis is concerned, their localization in CNS, pituitary and adrenal gland suggested an action of these receptors at different levels. PBR agonists have been shown to increase CRH and ACTH release from rat hypothalamus and pituitary, respectively (38), sug-

Table 1 - *Influence of benzodiazepine (BDZ) receptors on anterior pituitary hormones.*

	GABA/BDZ receptors	PB receptors
ACTH	↓ (20-23, 40-48, 58-69, 71, 73-79, 80, 89) = (49-52)	↑ (24, 25, 38)
GH	↑ (32, 62, 63, 90-94, 101-104, 108, 111, 136) ↓ (68, 100, 105, 106) = (95-99, 135)	↑ (39)
Gonadotropins	↓ (26, 117-121) ↑ (59, 88, 108, 122)	↓ (108)
PRL	↑ (62, 126, 136) ↓ (127-130) = (116,125,135)	↓ (127)
TSH	= (32, 102, 127, 130, 137) ↓ (109)	No data available

↓ inhibitory effect, ↑ stimulatory effect, =no effect. Notice: GABA/BDZ receptors are the central BDZ receptors; PB receptors are peripheral BDZ receptors (PBR) that have been shown also in the central nervous system.

gesting a peculiar stimulatory role of PBR in the modulation of HPA axis. On the other hand, although PBR activation stimulates steroidogenesis in the adrenal gland, some studies showed an inhibitory effect of diazepam on ACTH-induced steroidogenesis in animals (24). In agreement with this hypothesis, we have demonstrated that AL significantly blunts the adrenal response to low ACTH doses in humans (76). However, it is noteworthy that endozepines play a key role in the stimulation of steroidogenesis in the adrenal gland by activating PBR (24, 25). Based on this evidence, it has been hypothesized that DBI may be involved in the long-term trophic effect of ACTH on adrenal gland though it unlikely mediates the short-term adrenal response to ACTH (25). Taken all together, these data show how complex are effects of PBR activation on HPA axis and suggest that their activation has different effects depending on different receptor localization and/or different ligands.

From a clinical point of view, it is well known the peculiar efficacy of AL in the management of panic disorders and depression, probably due to its marked inhibiting effect on HPA axis (21-23). On the other hand, it has to be pointed out that BDZ may be a useful tool to investigate GABAergic as well as HPA activity in some pathophysiological conditions. To this regard, it has been demonstrated that BDZ do not modify ACTH hypersecretion in patients with Cushing's disease (77, 89), suggesting that GABA is unable to influence corticotrope secretion in this condition. Moreover, we have previously shown that AL possesses a less potent blunting effect on ACTH and F response to hexarelin in obesity than in normal subjects (89), suggesting that the inhibitory influence of BDZ/GABA on the ACTH-releasing activity of GH secretagogues is impaired in obesity.

Finally, the data above reported clearly indicate that the effects of BDZ on HPA function should be taken in account when endocrinological assessment is performed in patients with suspected impaired HPA activity; moreover, when BDZ treatment has to be started in patients with anxiogenic disorders, caution in the use of these drugs, especially AL, should used in those patients with suspected hypoadrenalism (Table 1).

BDZ AND SOMATOTROPE FUNCTION

The first studies focusing on the activity of BDZ on GH secretion reported controversial results. Diazepam had been reported to significantly increase GH release in normal subjects in a dose-dependent manner (90-92) and similar results were found using other BDZ molecules (93). Moreover, evidence that flumazenil, an inverse agonist of the GABA/BDZ binding site, significantly inhibited nocturnal GH release suggested a tonic stimulatory influence of GABA/BDZ on GH secretion (94).

Other Authors failed to confirm a positive effect of BDZ on somatotrope function; neither diazepam affected either morning or nocturnal spontaneous GH release and nor BDZ, such as triazolam, flurazepam or bretanezil had any significant effect (95-99). Even an inhibitory effect of BDZ on somatotrope secretion was reported after administration of the BDZ agonist midazolam (100). As marked inter-subject variability in the GH response to BDZ has been found (101, 102), this could probably explain the controversial data reported above.

Clear stimulatory effect of BDZ on GH secretion has been demonstrated in the studies addressing the effects of AL. In fact, it clearly increases GH secretion in both rats and humans, showing in the

latter dose-related effect (62, 63, 103, 104). When evaluated in equipotent doses in rats, AL showed higher GH-releasing effect than diazepam, once again indicating the peculiarity of this benzodiazepine (104).

Although BDZ possess *per se* stimulatory effect on spontaneous GH secretion, it is remarkable that they are paradoxically able to blunt GH response to some provocative stimuli. AL, in fact, blunts the GH response to hexarelin, a synthetic GH secretagogue (68) as well as to meta-chlorophenylpiperazine, a serotonin receptor agonist (105) (Fig. 2). Moreover, diazepam had been shown able to reduce the GH response to L-DOPA and apomorphine in normal subjects (106). The explanation of the paradoxical effects of BDZ on somatotrope secretion (stimulatory on spontaneous but inhibitory on stimulated GH secretion) is still unclear but, anyway, indicates a tight interplay between GABA/BDZ and the major neural systems controlling somatotrope function. In agreement with this hypothesis, it has also been demonstrated that GABA, which positively influences spontaneous GH secretion, blunts the GH responsiveness to insulin-induced hypoglycemia (107).

As far as the mechanisms underlying the BDZ effects on GH secretion are concerned, it has been shown that GABA antagonists are able to inhibit GH response to BDZ while BDZ receptor antagonists are able to reverse the effects of both BDZ agonists and inverse agonists on GH secretion (108). Thus, this indicated that BDZ actually act through GABA/BDZ receptor activation to modulate GH release.

The influence of BDZ on GH secretion is likely to be mediated at the hypothalamic and/or suprahypothalamic level. This hypothesis based on the evidence that central BDZ receptor ligands show little or no effect on GH release in both normal and tumoral rat somatotroph cells *in vitro* (39, 109) and that BDZ treatment does not modify the GH response to GHRH in either normal subjects or patients with panic disorder (110).

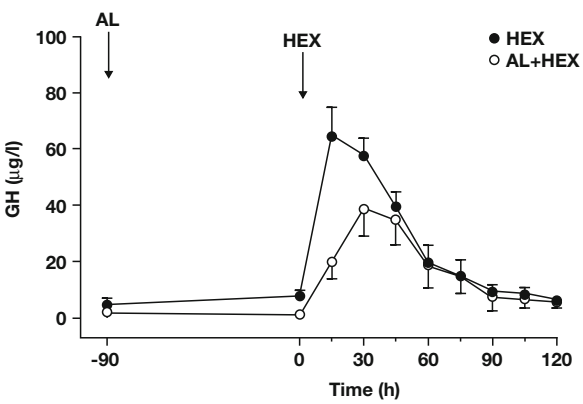

Fig. 2 - *Mean (±SE) GH responses to Hexarelin (HEX, 2.0 µg/kg i.v. at 0 min) preceded by either placebo or alprazolam (AL, 0.02 mg/kg, orally, at -90 min) in normal subjects.*

AL-induced GH rise is blocked in rats by yohimbine and idazoxane, two $\alpha 2$ antagonists (104), suggesting that the effect of this BDZ on GH release could involve the $\alpha 2$ adrenoceptor activation, although AL does not bind $\alpha 2$ receptors (63). A dopaminergic mediation of BDZ effect on GH release has also been suggested, as the GH response to diazepam is significantly reduced by dopaminergic antagonists (111). Finally, the evidence that both GABA and BDZ inhibited somatostatin release from some rat central areas *in vitro* (112) led to hypothesize a somatostatin-mediated action of GABA/BDZ system in the modulation of somatotrope function. However, against this hypothesis is the evidence that neuroactive substances acting via inhibition of somatostatin release show potentiating effect on GHRH-induced GH rise (113), while BDZ do not demonstrated any effect on this response in humans (110).

Although direct effect of BDZ on somatotrope cells had not been found by some Authors (39,109), BDZ agonists have been shown to be able to potentiate the GH rise induced by GABA agonists in rat pituitary (32), suggesting direct stimulatory modulation of BDZ on GH release. Moreover, GABA immunoreactivity has been found in rat somatotrope cells, suggesting an autocrine and/or paracrine effect of GABA on somatotrope function (114). Interestingly, TRH-induced GH rise has been reported to be inhibited by BDZ pretreatment in rat pituitaries, as consequence of the competition for pituitary TRH receptors (32, 115). This evidence indicates that BDZ may also negatively modulate GH release in the pituitary gland by acting on receptors other than GABA/BDZ ones. In this context, PBR seem involved in the BDZ modulation of somatotrope function at pituitary level; in fact, the exposure of tumoral GH3 cells to a PBR agonist increases GH secretion (39), suggesting a stimulatory influence of these binding sites on GH secretion.

The possibility that the GH-releasing activity of BDZ has some clinical impact seems very unlikely. From the diagnostic point of view, the great intraindividual variability in the GH response to BDZ in normal subjects strongly reduces their reliability as provocative stimuli to evaluate the secretory capacity of somatotrope cells in the suspect of GH deficiency (101, 102, 116) (Table 1).

BDZ AND GONADOTROPE, LACTOTROPE AND THYROTROPE FUNCTION

Gonadotrope function

A BDZ influence on the activity of the hypothalamic-pituitary-gonadal axis has been shown and it is mainly mediated by GABA/BDZ receptor binding. GABA plays a major role in the control of gonadotropin release, by acting at different levels. Besides the extensive network of GABA-containing fibers in the hypothalamus, which are likely to interact with GnRH-secreting neurons, (34, 35), GABAergic receptors have been demonstrated in the pituitary gland, which probably mediate a direct influence of GABA on gonadotrope cells (11, 88).

The activation of the GABA-A receptor complex by GABA, GABA agonists, barbiturates as well as endogenous and synthetic BDZ inhibits GnRH gene expression in rat medial preoptic area (117-119) and that this effect is completely antagonized by GABA-A/BDZ antagonists (26, 117-119). Thus, GABA/BDZ receptor activation is likely to exert a tonic inhibitory influence on GnRH-secreting neurons. On the other hand, direct effect of GABA on gonadotrope function has also been described (88). The presence of either inhibitory or excitatory influence of GABA receptors on gonadotrope cells has been shown; the activation of the latter ones potentiates the effect of GnRH on the intracellular mechanisms leading to gonadotropin secretion (88). Thus, it has been hypothesized that the stimulatory effect of BDZ/GABA on gonadotrope function may reflect a pituitary action while the inhibitory influence could reflect mediation by central BDZ/GABA receptors (108).

In agreement with the hypothesis of a dual effect of GABA/BDZ on gonadotrope function, some *in vivo* studies demonstrated an inhibitory effect of BDZ on LH release (120, 121), while other Authors showed a positive effect of BDZ on LH secretion (59, 108). Moreover, some studies in humans showed that AL increases LH pulse amplitude in normal women in early follicular phase and restores LH pulsatility in women with hypothalamic stress-related amenorrhea (122). As the stress-induced CRH rise seems involved in the pathogenesis of hypothalamic amenorrhea, it has been suggested that AL would prevent the inhibition of LH release in hypothalamic amenorrhea through reduction of the stress-induced hyperactivation of the HPA axis (122); this would point out the importance of some BDZ, such as AL, in stress-related pathophysiological conditions.

While there is clear evidence for the involvement of PBR in the synthesis of gonadal steroids, few data are so far available on the effects of these receptors on gonadotropin secretion. It has been demonstrated that PBR activation is followed by the inhibition of gonadotrope function in animals (108). On the other hand, a modulatory role of GnRH, PRL and gonadal steroids on both central and peripheral BDZ receptor expression has been found in different tissues. In fact, hypothalamic, pituitary and adrenal PBR expression is reduced during pregnancy and lactation in rats (123), as well as in platelets of pregnant women (124), thus suggesting a negative influence of hypothalamic-pituitary-gonadal axis as well as of PRL on PBR function. On the contrary, the hormonal changes occurring during pregnancy were found associated with an increase of PBR density in ovary and uterus (123), suggesting a tissue-dependent effect of the hypothalamic-gonadal axis on the expression of these receptors. In agreement with this hypothesis, BDZ receptor expression was increased in the hippocampus but decreased in both hypothalamus and pituitary during pregnancy in rats (123).

Taken all together, the data above mentioned indicate a complex and differentiated modulatory influence of BDZ on the activity of hypothalamic-pituitary-gonadal axis, which, in turn, modulates in different ways and at different levels the activity of BDZ themselves (Table 1).

Lactotrope function

The influence of BDZ on PRL secretion is still controversial: stimulatory, inhibitory or lacking influences have, in fact, been reported.

Very high diazepam doses have been found to stimulate PRL release in schizophrenic patients but other BDZ showed slight or no effect on either basal or stimulated PRL release (116, 125, 126). On the contrary, a stimulatory action of both acute and chronic AL administration on basal PRL levels has been shown in normal subjects as well as in patients with panic disorder, respectively (62,126).

Evidence for BDZ inhibitory effect on both basal and stimulated PRL secretion has been reported in both animals (127-129) and humans (130).

The mechanisms by which BDZ modulate PRL secretion are likely to involve GABAergic mediation. A dual effect of GABA on PRL release has been demonstrated: one stimulatory, likely to occur at hypothalamic level, and one inhibitory, which seems to be mediated at pituitary level. In agreement with the central positive action of GABA on PRL release is the evidence that the injection of GABA-A agonists in the hypothalamus induces a clear rise in PRL levels in rats (131). This effect seems mediated by the inhibition of the tubero-infundibular dopaminergic system; important GABA-dopamine interactions in CNS, namely in the hypothalamus, are pre-

sent and inhibitory action of GABA and BDZ on dopaminergic neurotransmission has been demonstrated (132, 133).
It had been hypothesized that PBR could mediate the inhibitory effect of BDZ on PRL secretion at pituitary level. Actually, PBR ligands display pituitary BDZ binding with more affinity than central BDZ/GABA agonists (1). However, more recent data showed that PBR ligands have no effect on PRL release from pituitary gland, while central BDZ/GABA agonists inhibit both basal and stimulated PRL secretion *in vitro*, thus indicating that the GABA/BDZ receptor complex mediates the pituitaric effects of BDZ on PRL release (127).
Finally, direct positive influence of GABA on PRL release has also been shown, indicating a complex influence of GABA/BDZ on PRL secretion mediated at different levels (134).
From a clinical point of view, the effect, if any, of chronic BDZ treatments on PRL levels and/or the effects of BDZ in conditions of hyperprolactinemia are scanty and do not indicate a strong influence of these substances on the mechanisms deeply controlling PRL (130, 135, 136); thus, further studies are needed to clarify the real physiological and clinical impact of BDZ in the control of lactotrope function (Table 1).

Thyrotrope function

The influence of BDZ on thyrotrope function does not appear remarkable but data available so far are scanty. BDZ have been reported to inhibit TSH responsiveness to pharmacological stimulations in animals (130). Moreover, BDZ have been reported to be able to blunt the TSH response to TRH in rat pituitaries through a competition for the TRH receptor (109). On the contrary, other *in vitro* studies did not confirm the inhibitory effect of BDZ on either basal or TRH-stimulated TSH release (32, 127).
Studies in humans showed that neither acute nor protracted BDZ administration modified basal or TRH-stimulated TSH secretion in normal subjects or in patients with anxiogenic disorders, respectively (102, 130, 137). Nevertheless, it is of interest that thyroid hormones seem to influence the binding and function of the brain GABA/BDZ receptor complex, suggesting an interplay between the hypothalamo-pituitary-thyroid axis and GABA/BDZ activity in the CNS (138), (Table 1).

CONCLUSIONS

Although for a long time BDZ have been shown to influence hormonal secretions in animals and humans, the discovery of specific binding sites for BDZ (GABA/BDZ and PBR) led to hypothesize the existence of natural ligands with a physiological role. The isolation of the family of endozepines, natural ligand of both GABA/BDZ and PBR, allowed to demonstrate the existence of a new system deeply involved in many central, neuroendocrine and peripheral actions specifically mediated by GABA/BDZ or PBR activation. As classical BDZ mainly bind GABA/BDZ binding sites, their effects are likely to reflect the GABA/BDZ-mediated actions of endozepines. However, unlike classical BDZ, endozepines strongly control the processes of steroidogenesis in all steroidogenetic tissues through PBR activation. In CNS, PBR activation stimulates the synthesis of the neurosteroids, which, in turn, modulate GABAergic neurotransmission by acting on GABA/BDZ receptors. So, a tight interplay between GABA, BDZ and neurosteroids became apparent in the brain. Among the neuroendocrine activities of BDZ, the inhibitory effect on the HPA function seems to be the most important. Noteworthy, among the various BDZ, AL showed the most marked inhibitory effect on both spontaneous and stimulated HPA activity, in agreement with its peculiar efficacy in panic disorders and depression, where the HPA hyperactivation seems to be involved in pathophysiology of the disease. For these reasons, BDZ, especially AL, may be a good tool to study HPA neuroregulation. Moreover, the effects of these substances have to be taken into account when endocrinological investigations are performed as well as before treating patients with suspected adrenal impairment.

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