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REVIEW ARTICLE

Current Perspectives for the use of Gonane Progesteronergic Drugs in the Treatment of Central Hypoventilation Syndromes

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Abstract: ***Background*:** Central alveolar hypoventilation syndromes (CHS) encompass neurorespiratory diseases resulting from congenital or acquired neurological disorders. Hypercapnia, acidosis, and hypoxemia resulting from CHS negatively affect physiological functions and can be life-threatening. To date, the absence of pharmacological treatment implies that the patients must receive assisted ventilation throughout their lives.

***Objective*:** To highlight the relevance of determining conditions in which using gonane synthetic progestins could be of potential clinical interest for the treatment of CHS.

***Methods*:** The mechanisms by which gonanes modulate the respiratory drive were put into the context of those established for natural progesterone and other synthetic progestins.

***Results*:** The clinical benefits of synthetic progestins to treat respiratory diseases are mixed with either positive outcomes or no improvement. A benefit for CHS patients has only recently been proposed. We incidentally observed restoration of CO_2 chemosensitivity, the functional deficit of this disease, in two adult CHS women by desogestrel, a gonane progestin, used for contraception. This effect was not observed by another group, studying a single patient. These contradictory findings are probably due to the complex nature of the action of desogestrel on breathing and led us to carry out mechanistic studies in rodents. Our results show that desogestrel influences the respiratory command by modulating the $GABA_A$ and NMDA signaling in the respiratory network, medullary serotoninergic systems, and supramedullary areas.

***Conclusion*:** Gonanes show promise for improving ventilation of CHS patients, although the conditions of their use need to be better understood.

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1. INTRODUCTION

Breathing is an essential part of life that depends on a rhythmic command from a brainstem neuronal network [1-3]. Within this neuronal network, two groups of medullary neurons play an important role, the pre-Botzinger complex, which drives inspiration [1, 2, 4], and the parafacial respiratory group (pFRG), which controls pre-inspiratory and expiratory activities [1, 2, 5]. In addition, a recently discovered group of neurons, called the post-inspiratory complex, has been proposed as a post-inspiration generator [6]. Breathing displays a strong variability allowing the respiratory network to accurately and quickly react to even small changes in en-

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vironmental and behavioral conditions [7-12]. In particular, the central respiratory drive is finely regulated to be adjusted to blood variations of O_2, CO_2, and pH, detected primarily by carotid body peripheral and ventral medullary surface central chemoreceptors [13]. Ventral medullary surface chemoreceptors for CO_2/H^+ are included in an entity called the retrotrapezoid nucleus that overlaps with the pFRG and expresses the *paired-like homeobox 2B* (*PHOX2B*) [5, 14]. They are considered to be the principal CO_2/H^+ chemosensory cells [14-16]. Neurons from other central structures have also been shown to be CO_2/H^+ chemosensitive and stimulate the central respiratory drive, such as neurons from the caudal hypothalamus in the diencephalon, periaqueductal gray matter in the mesencephalon, the locus coeruleus in the pons, and raphe nuclei of the medulla oblongata [17-24].

Central alveolar hypoventilation syndromes (CHS) are neurorespiratory diseases resulting from impairment of the neuronal network that controls respiratory rhythm generation and its modulation by peripheral or central afferent inputs [25]. Insufficient ventilation in CHS leads to an increase in $PaCO_2$ and a decrease in PaO_2, only or mostly during non-rapid eye movement sleep, a state during which the central respiratory drive depends predominantly on chemosensitive afferents. The diagnostic criteria of CHS are *i*) persistent central hypoventilation with a $PaCO_2 > 60$ mmHg during sleep, with or without apnea, detected by polysomnography while the patient spontaneously breathes room air, *ii*) lack of ventilatory response to inhaled CO_2, *iii*) and the absence of primary lung, neuromuscular, or cardiac disease [26, 27]. Hypercapnia, acidosis, and hypoxemia, resulting from central hypoventilation, lead to negative short and long-term effects. These include neural damage that induces deleterious effects on cardiovascular function and neurocognitive development, leading to a risk of sudden death and profoundly affecting neurocognitive outcome and long-term quality of life [28-33]. CHS encompass several pathological conditions. They may be congenital, such as the most documented CHS, the Congenital Central alveolar Hypoventilation Syndrome (CCHS) or the Late Onset Hypoventilation Associated with Hypothalamic Dysfunction Syndrome. They may also be acquired, such as through ischemic or hemorrhagic brainstem lesions or in the case of the Obesity Hypoventilation Syndrome.

Some drug classes are broadly considered to be conventional respiratory stimulants, among them natural progesterone and synthetic progestins [34-43]. However, the prescription of respiratory stimulants and physiological conditions in which plasma levels of natural progesterone are high (pregnancy) have failed to improve the ventilation of CHS patients [44-48]. The absence of available pharmacological treatment results in CHS patients receiving life-long treatment by mechanical ventilation, at least during sleep, either with positive pressure ventilation *via* a tracheostomy or a mask, with phrenic nerve pacing, or with negative pressure ventilation *via* a cuirass [49]. These diseases present a true handicap for patients and a decrease in the quality of life [32, 33]. This unsatisfactory therapeutic solution, due to the dependence of patients on the machine, underlines the interest in developing pharmacological alternatives. A recent serendipitous clinical observation has suggested that a specific family of progestin, the gonanes, has a beneficial effect in some CCHS patients [50]. This first observation has led to experimentation in rodents to characterize the modes of action of these molecules on ventilatory control [51, 52].

The aim of this review is to first present the modes of action of natural progesterone, its derivatives, and synthetic progestins on the central nervous system (CNS) and then summarize animal and clinical data concerning progesteronergic drugs and the central control of breathing with the objective to stimulate interest in gonane progestin drugs as a potential treatment modality for CHS patients.

2. NATURAL PROGESTERONE, ITS DERIVATIVES, AND FAMILIES OF SYNTHETIC PROGESTINS HAVE VARIOUS MODES OF ACTION ON THE CNS

For many years, the potential role of natural progesterone, its metabolites, and synthetic progestins on the CNS has been of clinical interest. Natural progesterone and its metabolites can be considered to be neuroactive steroids: *i)* their lipophilicity allows a small fraction to cross the blood-brain barrier [53, 54] and *ii)* natural progesterone and its metabolites can be synthesized by cells of the CNS *de novo*, independently of peripheral steroidogenesis, making them potential neurosteroids [55, 56]. All synthetic progestins have in common the ability to reproduce the biological effect of natural progesterone by binding, at least in part, to the same receptors as the endogenous molecule. They are commonly used in the clinic and are derived either from testosterone (19-nortestosterone derivatives) or progesterone (17-hydroxyprogesterone and 19-norprogesterone derivatives) (Fig. **1**). Each subgroup of derivatives contain several classes of progestin: the gonane and estrane classes belong to the 19-nortestosterone derivatives, and the pregnane and norpregnane classes belong to the 17- hydroxyprogesterone and 19-norprogesterone derivatives, respectively [57]. Beyond the progestogenic effects of all progestins, understanding of the biological activities of progestins requires extensive knowledge of the inherent characteristics of each compound. That includes knowledge of their chemical structure, metabolism, depending on the route of administration, pharmacodynamics, and bioavailability, depending on storage sites in the body and binding to serum proteins [58]. Natural progesterone, its metabolites, and progestins all have neuronal functions due to their binding to cognate intracellular and membrane receptors or receptors belonging to other neurotransmitters.

2.1. Genomic Effect of Natural Progesterone, and Progestins

Nuclear progesterone receptors (PR) mediate the genomic actions of natural progesterone, its metabolites, and synthetic progestins by transregulating the expression of genes containing a Progesterone Responsive Element (PRE) sequence in their promoter [59]. Upon ligand binding, the subsequent conformational changes of the receptors lead to chaperone protein dissociation [60, 61]. The ligand/receptor complex then dimerizes before being translocated into the nucleus, where it interacts with the PRE sequence of the target promoters [62, 63]. Within the CNS, natural progesterone, its metabolites, and synthetic progestins bind to the pre-

Figure 1

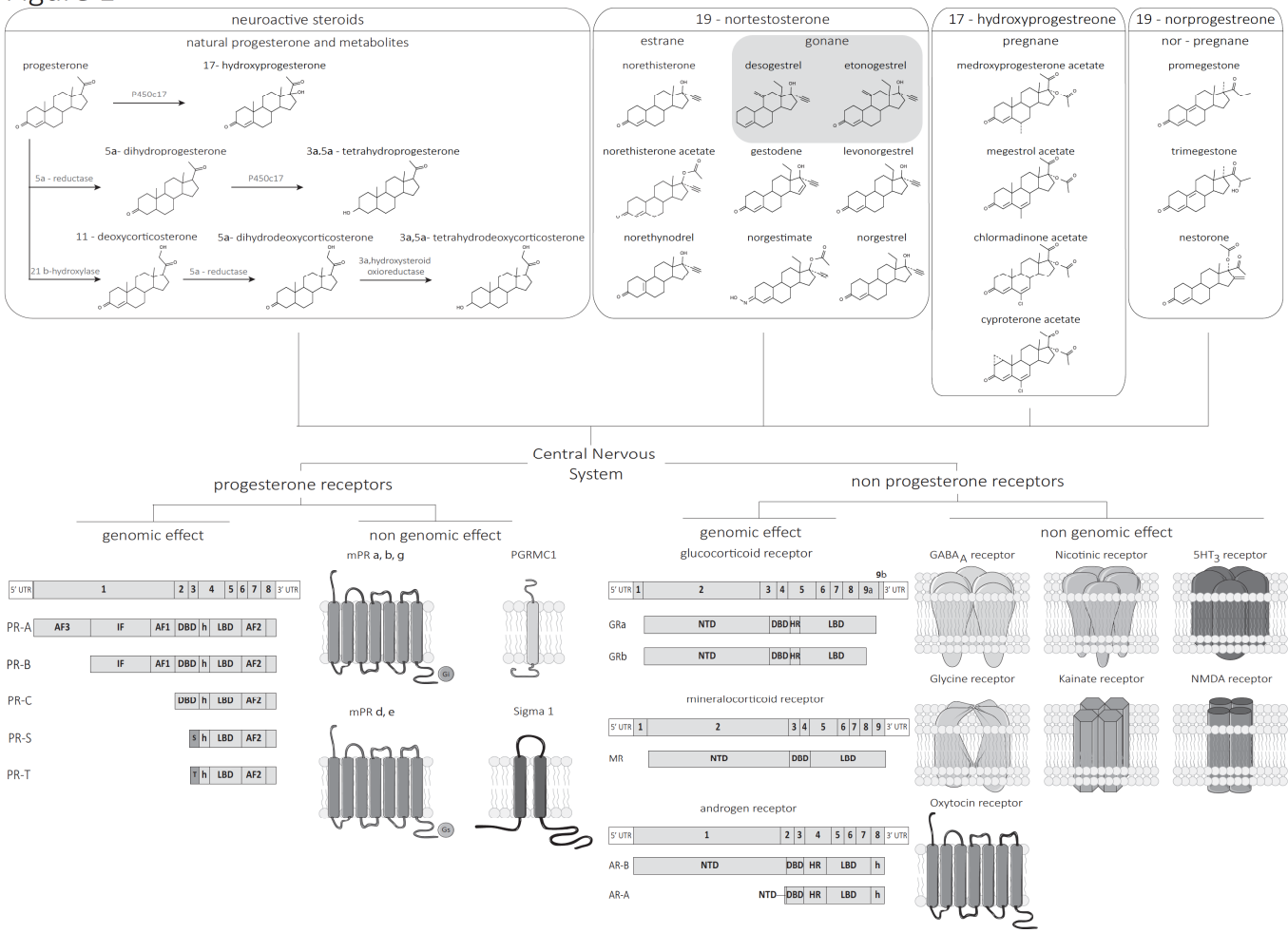

Fig. (1). Natural progesterone, progesterone derivatives, and families of synthetic progestin and their genomic and non-genomic modes of action within the CNS. The upper part of the figure shows the chemical structures of natural progesterone, its derivatives, and synthetic progestins. The enzymes required for the biosynthesis of natural progesterone and its derivatives are indicated. The two members of the gonane family, desogestrel and etonogestrel, which are the focus of this review, are highlighted by a gray rectangle. The lower part of the figure shows the receptors targeted by neuroactive steroids and synthetic progestins. The left-hand side shows the cognate nuclear and membrane receptors of progesterone that mediate their genomic and non-genomic effects, respectively. The right-hand side shows the receptors which belong to other neurotransmitter systems that are targets of neuroactive steroids and synthetic progestins. For nuclear receptors, the canonical mature mRNA is represented with its 5' and 3' untranslated regions. The scheme of the canonical mature mRNA is composed of boxes containing numbers which correspond to the exon from which they were transcribed. The various protein isoforms translated from the mature mRNA are represented with their functional domains located just below the representation of the mature mRNA. For the nuclear progesterone receptor isoforms PR-S and PR-T, boxes S and T represent nucleotide sequences that are translated from intronic-exons not represented in the canonical mature mRNA. Abbreviations: steroid 17-alpha-hydroxylase/17,20 lyase precursor (P450c17), untranslated region (UTR), activation function (AF), inhibition factor (IF), DNA binding domain (DBD), nuclear location signal (h), ligand binding domain (LBD), intronic-exons (T and S), N-terminal domain (NTD), nuclear progesterone receptor (PR), nuclear glucocorticoid receptor (GR), nuclear mineralocorticoid receptor (MR), nuclear androgen receptor (AR).

dominant isoforms PR-A and PR-B obtained using alternative promoters. In addition, PR-C, PR-S, and PR-T isoforms have also been identified (Fig. **1**) [59, 64]. Each isoform has distinct transcriptional activities of which the mechanisms have not been fully elucidated [59, 64]. PRs are found in brainstem structures involved in the central respiratory drive: the ventrolateral region of the medulla oblongata [65], nucleus of the solitary tract [66], hypoglossal nucleus [65, 67], locus coeruleus [68], and parabrachial nucleus [65]; in the diencephalon [69-72], and in the telencephalon [71, 72]. Natural progesterone, its metabolites, and synthetic progestins can also act through nuclear receptors other than the PR: the glucocorticoid and mineralocorticoid receptors (Fig. **1**) [58]. In addition, several synthetic progestin members of the 17-hydroxyprogesterone derivatives (megestrol acetate and medroxyprogesterone acetate) and almost all 19-nortestosterone derivatives (estrane and gonane family) have been shown to have androgenic activity by binding to nuclear androgenic receptors with varying affinity [58].

In addition to the relatively well known interactions of natural progesterone, its derivatives and synthetic progestins with nuclear receptors, these steroid drugs may act by other ways.

2.2. Non-genomic Effects of Natural Progesterone, its Metabolites and Synthetic Progestins

Evidence has emerged that neuroactive steroids have modulatory effects on neuronal functions through a non-genomic pathway, as studies have shown their ability to initiate intracellular signaling pathways and the recruitment of second messengers in a time window from a few milliseconds to a second [73-75]. It is therefore unlikely that these effects are due to conventional genomic mechanisms involving gene transcription and protein synthesis, which are relatively slow processes taking from several minutes to hours.

2.2.1. Membrane Receptors of Progesterone

Progesterone membrane receptors (mPR) contain seven transmembrane domains and belong to the adipoQ family [76-78]. The mPR family is composed of 11 members subdivided into three classes [79]. Only class II mPR, which has five members, are sensitive to progesterone [80]. mPRα, mPRβ, and mPRγ are coupled to an inhibitory G protein [81-83], whereas mPRδ and mPRε, which have a higher affinity for progesterone, [80, 81, 84, 85] are coupled to a stimulatory G protein (Fig. **1**) [84]. The biological effects which result from the binding of progesterone to these receptors are yet to be determined [86]. Several studies have described the distribution of proteins and mRNAs encoding various isoforms within the CNS [84, 87, 88]. These receptors, expressed preferentially in neurons [88], are present in the spinal cord, brainstem, cerebellum, hypothalamus, hippocampus, and cerebral cortex [84, 87-90]. mPRδ is, to date, the only member identified to be specific to the CNS [84].

Binding of natural progesterone to progesterone receptor membrane component 1 (PGRMC1) is rapid, selective, and reversible [91, 92]. The functional relevance of this interaction has only just started to be elucidated and includes the activation of extracellular signal-related kinase pathways [86, 93]. Several studies have demonstrated the presence of mRNA encoding this single-transmembrane protein in brainstem and structures involved in respiratory control, such as the pre-Botzinger complex [94], the nucleus of the solitary tract, periaqueducal gray matter, and the caudal hypothalamus [85, 94].

The sigma type 1 receptor, initially misnamed as "opioid" receptor [95, 96], is located in the mitochondria-associated endoplasmic reticulum membrane and translocates to the plasma membrane upon fixation of agonists or stressors [97]. Two subtypes have been described called the sigma 1 and sigma 2 receptors [98]. The sigma 1 receptor has been clones, whereas the sigma 2 receptor has not [99]. Moreover, it has been suggested that the sigma 2 receptor is the previously described PGRMC1; further studies are required to clarify its final identification [100]. Interactions have been experimentally demonstrated between the sigma 1 receptor and dopamine and muscarinic acetylcholine receptors [97, 101], as well as many endogenous ligands [96, 97, 102]; among them, natural progesterone is one of the most potent antagonists described [96, 101, 103, 104]. Natural progesterone binds competitively at the orthosteric site and is therefore considered to be an endogenous ligand [96, 101, 104]. Sigma 1 receptor mRNA and protein have been detected by *in situ* hybridization and immunohistochemistry in the diencephalon, for instance in the hypothalamus, as well as in the brainstem: periaqueducal gray matter, locus coeruleus, hypoglossal nucleus, and medullary raphe nuclei [102, 105, 106]. The physiological significance of these interactions are still unknown and require further studies [86].

2.2.2. Membrane Receptors to Endogenous Chemical Compounds other than Natural Progesterone

There is now considerable evidence that natural progesterone, its metabolites, and synthetic progestins are allosteric modulators of ligand-gated ion channels belonging to the Cys-loop family: $GABA_A$, nicotinic acetylcholine, glycine, and $5HT_3$ receptors, as well as the glutamatergic ion channel receptors, the NMDA and kainate receptors (Fig. **1**). These receptors are all distributed throughout the CNS from the telencephalon to the spinal cord [54, 107-117].

The modulation of $GABA_A$ receptor activity by natural progesterone, its metabolites, and synthetic progestins is well documented. The neuroactive steroid binding site, responsible for the modulation of $GABA_A$ receptor, is located at the highly conserved transmembrane domain of the α subunit [118]. Natural progesterone, its metabolites and synthetic progestins have a variety of effects on $GABA_A$ receptors that have be linked to their chemical structure. The structure-activity relationship is highly complex and relies on several chemical characteristics of the steroids including *i)* the α/β-configuration of the constituents [119, 120], *ii)* the trans- or cis-configuration in the backbone structure [121], *iii)* the presence of donor or acceptor hydrogen-bond at specific position [122-124], even if it does not appear to be systematically required [125, 126] and *iv)* the substitution or addition of groups in certain positions of the steroid molecules [127-129]. For instance, dehydroepiandrosterone, is a negative allosteric modulator of $GABA_A$ receptors whereas 3α5α tetrahydroprogesterone (allopregnanolone), 5β-pregnan-3α-ol-20-one (pregnanolone) and natural progesterone are positive allosteric modulators of $GABA_A$ receptor [130]. Furthermore, some data of the literature suggest that $GABA_A$ receptors modulation by neurosteroid may be influenced by the subunit composition of $GABA_A$ receptors [54, 131, 132]. $GABA_A$ receptor presents a large panel of structural organization because of its heteropentameric combination. To date, at least 19 $GABA_A$ receptor subunit genes (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, θ, π, and ρ_{1-3}) have been identified by sequencing the Human genome [133, 134]. However, only a few dozen combinations seem to exist *in vivo* suggesting a selecting process in the $GABA_A$ receptor assembling [133]. Although not fully understood to date, heterologous expression systems suggest that the presence of δ subunit confers to the $GABA_A$ receptor an increased sensitivity to pregnanolone [135], allopregnanolone [136], and 3α5α tetrahydrodeoxycorticosterone (THDOC) [137, 138]. Additionally, the modulation of $GABA_A$ receptors activity by neurosteroid may also depend on the phosphorylation state of $GABA_A$ receptors [139] and on the neurosteroid concentration according to the law of mass action [130, 136, 140]. Finally, expression of $GABA_A$

receptors is ubiquitous in the CNS, whereas the expression of steroidogenic enzymes is region specific, suggesting that neurosteroid/$GABA_A$ receptor interactions are highly selective and that neurosteroids may specifically interact with distinct pools of $GABA_A$ receptors [54, 132].

The established link between the chemical structure of neurosteroids and their effect on $GABA_A$ receptor does not allow prediction of their effect on other ligand-gated ion channels of the Cys-loop family. Indeed, natural progesterone is a non-competitive negative allosteric modulator of the nicotinic acetylcholine receptor at micromolar concentrations [141-145], as well as A-ring reduced metabolites of progesterone 5α-dihydroprogesterone and allopregnanolone [143, 146]. In contrast to $GABA_A$ receptor, the composition of nicotinic acetylcholine subunits has less influence on neuroactive steroid-induced allosteric modulation, as natural progesterone induces a similar effect, regardless of the subunit composition [143, 145]. Experiments made with natural progesterone that was bound or not to albumin, a water-soluble compound that does not cross the plasma membrane, showed that both bound and unbound progesterone were able to exert negative allosteric modulation of nicotinic acetylcholine receptor activity, suggesting an extracellular site of action [142, 145].

Comparatively little is known about the influence of neuroactive steroids on glycine receptors. Wu *et al*. have shown that natural progesterone and two of its metabolites (11-deoxycorticosterone and 17-hydroxyprogesterone, which differ from natural progesterone by the presence of a hydroxyl group at C21 and at C17, respectively) act on glycine receptors as negative allosteric modulators [147]. 11-deoxycorticosterone has similar activity whereas 17-hydroxyprogesterone is less potent than natural progesterone. Allopregnanolone, which has a hydroxyl group at C3, has no effect, suggesting that the structure-activity requirements for steroid interactions are different for $GABA_A$ and the glycine receptors.

Other studies have shown that neuroactive steroids can modulate cation flux through the ionotropic channel 5-HT_3 [148-150]. Natural progesterone [148-150], its metabolites allopreganolone [148] and THDOC [150], and the synthetic progestin cyproterone acetate [150], a member of pregnane family, can all exert negative allosteric modulation of the $5HT_3$ receptor. The interaction between natural progesterone and the $5HT_3$ receptor may occur at the receptor-membrane interface or at an extracellular site, as natural progesterone bound to the hydrophilic compound albumin exerts the same functional allosteric activity as free progesterone and it binds to membranes of HEK 293 cells expressing the 5-HT_3 receptor [148].

Many studies have examined the effect of pregnenolone sulfate and its analog pregnanolone sulfate on the glutamatergic ionotropic receptors, the NMDA and kainate receptors [151-155]. Pregnenolone sulfate appears to positively and negatively modulate the NMDA and kainate receptor, respectively [151-155] and preganolone sulfate negatively modulates both receptors [154]. Only a few studies have shown an effect of neuroactive steroids derived from natural progesterone on NMDA and kainate receptors. Allopregnanolone weakly modulates NMDA currents [156] and induces increased release of neurotransmitters, which is blocked by specific NMDA antagonists [157]. Natural progesterone also rapidly and reversibly potentiates the kainate-induced current in a dose-dependent manner *via* interaction with an extracellular binding site [158]. Further studies are required to address the biological relevance of these effects.

2.2.3. G-protein-coupled Receptors to Endogenous Chemical Compounds other than Natural Progesterone

In addition to the modulation of ligand-gated ion channels, steroids are also able to modulate the activity of G-protein-coupled receptors (GPCRs) for other chemical compounds. For example, progesterone can disrupt oxytocin receptor-mediated signaling [159, 160]. It was proposed that natural progesterone specifically interacts with recombinant rat oxytocin receptors expressed on CHO cells and acts as a negative allosteric modulator by reducing the binding capacity of the specific ligand without affecting binding affinity [161]. This prevents oxytocin induced inositol phosphate production and calcium mobilization. This effect was not altered when natural progesterone was bound to albumin, excluding the possibility of a cytoplasmic site of action. Progesterone-induced repression of binding capacity has been postulated to be *i)* receptor specific, since natural progesterone had no effect on the closely related human oxytocin receptor and a related GPCR, vasopressin 1a receptor, and *ii)* steroid specific, as the progesterone metabolite 5β-dihydroprogesterone, which does not alter the binding capacity of rat oxytocin, negatively modulated the activity of the human oxytocin receptor [161]. This was the first study to highlight the existence of cross-talk between steroids and peptide-mediated signaling. Nevertheless, several observations do not support this assumption and it has been proposed that steroids have non-specific effects on oxytocin signaling [162-164]. Indeed, due to their structural properties, natural progesterone, its metabolites, and synthetic progestins are able to overload the plasma membrane, altering membrane fluidity and preventing the receptor from interacting with the G-protein [162, 163]. These regulatory mechanisms of progesterone shown in peripheral tissues probably occur in the CNS, where the oxytocin receptor is expressed [165, 166].

All the genomic and non-genomic actions of natural progesterone, its metabolites and synthetic progestins constitute a panel of action mechanisms as a base of their effects on the central respiratory drive.

3. FACILITATION OF THE CENTRAL RESPIRATORY DRIVE BY NATURAL PROGESTERONE AND SYNTHETIC PROGESTINS IN NON-PATHOLOGICAL SITUATIONS-MECHANISTIC APPROACHES

Studies in both human and animal models have established that natural progesterone, its derivatives and synthetic progestins exert a stimulatory effect on the baseline respiratory drive as well as during gas challenges, even if, in this last case, the literature on humans is somewhat contradictory. In this section, we present the main essential elements which have already been extensively reviewed [37, 46, 167, 168].

3.1. In Human, the Progesteronergic Systems Exert a Stimulatory Effect of the Respiratory Drive

Progesterone was first suggested to have a stimulatory effect on breathing in the early 1900s by Hasselbach *et al*. who reported a decrease in $PaCO_2$ during pregnancy, a physiological state in which plasma levels of progesterone are high [169, 170]. Later studies confirmed both the increase in baseline ventilation due to an effect on respiratory frequency [171] and hyperventilation and lower $PaCO_2$ during the luteal phase, when progesterone levels are higher than during the follicular phase [41, 172-174]. Similarly, breathing changes related to reduced synthesis of female sexual hormones during menopause confirmed their stimulatory effect, as ventilation is lower and $PaCO_2$ higher in postmenopausal than premenopausal women [36, 175]. In addition, administration of synthetic progestin to healthy men leads to increased ventilation and decreased $PaCO_2$ [34, 38, 176].

The influence of natural progesterone on O_2 and CO_2/H^+ chemoreception in human remains under debate. Indeed, in women, studies are in favor of a potentiation of the hypercapnic ventilatory response by the progesteronergic systems but others studies do not have this conclusion. First, an enhancement of the hypercapnic ventilatory response during luteal phase in comparison with follicular phase has been reported [177, 178]. Second, the analysis of breathing during a hyperoxic CO_2 rebreathing procedure in pregnant women revealed a correlation between pregnancy and an increased chemoreflex to CO_2. Indeed, plasma progesterone levels negatively correlated with a decrease of the central chemoreflex ventilatory recruitment threshold for CO_2, and tended to be positively correlated with increased sensitivity of the chemoreflex to CO_2 [171]. At the opposite, works reported that while women have been described to have an increased response to hypoxia during the luteal phase in comparison with follicular phase, no significant difference has been observed in response to hypercapnia between phases of the menstrual cycle [41, 179]. In addition, comparison of observations made on women and men suggest a gender effect for the respiratory response to chemoreflex challenge in humans because men have been described having a greater response to hypoxia or hypercapnia than women [171, 179]. Nevertheless, this gender difference seems to disappear after corrections for height and body mass index or O_2 consumption and CO_2 production [180, 181], whereas it was maintained in others [180, 181]. This is unexpected because of the recognized stimulatory effect of progesterone that is found in women and minimal in men, and illustrate the complexity of action of the progesterone on ventilation, for which the mechanisms are still poorly understood.

Based on the mechanistic known effects of progesteronergic systems, research for genomic and non-genomic action mechanisms has been carried out in animal models to explain the respiratory drive modulation by these steroids.

3.2. Progesteronergic Systems Stimulate Respiration Through Genomic and Non-genomic Effects

The stimulatory effect of natural progesterone on the respiratory drive has been confirmed in animal studies that were also designed to decipher the mechanisms, including localization of the effect, and the genomic and/or non-genomic actions by which natural progesterone, its derivatives or synthetic progestins stimulate breathing. To date, progesteronergic systems are thought to stimulate the respiratory drive by both directly affecting neurons of the respiratory network, including the neurons of the respiratory rhythm generators, and indirectly affecting structures and/or chemical systems that modulate the respiratory generators or other neurons of the respiratory network [37, 46, 167, 168]. In particular, progesteronergic systems are thought to influence breathing by acting mainly on the medulla and hypothalamus and by interfering with the modulation of breathing by serotonin. Intravenous administration or microinjection of progesterone into the nucleus of the solitary tract, a structure of the respiratory network located in the dorsal medulla, of anaesthetized and chemodenervated cats led to facilitation of phrenic nerve activity in both sexes that was abolished by pretreatment with RU486, an PR antagonist [182, 183]. These observations led the authors to conclude that the effect of progesterone on breathing, mediated through this structure, depends on genomic mechanisms. However, progesterone exposure induced a rapid reversion of the hypoxic neuronal response in 2-3 minutes in *ex vivo* medullary slices containing the nucleus of the solitary tract, suggesting that non-genomic mechanisms independent of the PR are involved [184]. Thus, the influence of mPR on the baseline respiratory drive and its adaptation to gas challenges has been recently evaluated in mice [185]. Small interfering RNA against mPR, leading to lower expression of mPR in the nucleus of the solitary tract, were applied to female and male mice. The occurrence of apnea in normoxia increased, the hypoxic ventilatory response was abolished, and the hypercapnic ventilatory response decreased, irrespective of the sex of the animals. Thus, natural progesterone or its derivatives may participate in the control of baseline respiration and hypoxia and hypercapnia chemoreflex loops *via* genomic and non-genomic mechanisms in the nucleus of the solitary tract. Another example of the action of natural progesterone on the medulla is its ability to stimulate hypoglossal motoneurons during long-term facilitation induced by serotonin-dependent intermittent hypoxia. Indeed, such long-term facilitation is enhanced during the diestrus phase, when the level of progesterone is high [186]. Published studies also suggest that the stimulatory effect of progesteronergic systems on breathing acts through the hypothalamus. The facilitating influence of natural progesterone is depressed in decerebrated cats, whereas it is maintained at a level similar to that in intact animals in decorticated cats in which the diencephalon is intact [182]. The authors of this study also reported that the increase in phrenic nerve activity following administration of natural progesterone was attenuated, but not abolished, in animals pretreated with anisomycin, an inhibitor of protein synthesis, or actinomycin-D, an inhibitor of RNA synthesis in the hypothalamus. These data suggest that at least part of the stimulatory effect of progesterone on breathing depends on gene expression in the hypothalamus. To date, there is no direct evidence that natural progesterone stimulates breathing *via* non-genomic mechanisms in this region of the brain. However, this possibility is supported by the presence of

mPRs in the hypothalamus [84, 87, 88]. Mechanistically, the action of progesterone may be similar to that of serotoninergic systems that are critically involved in the neural control of breathing [46, 187]. Indeed, the serotoninergic systems are sexually dimorphic at the level of respiratory motor nuclei, where fluctuating ovarian hormone levels are reflected by serotonin levels [46]. Similarly, exogenous administration of natural progesterone modifies the serotonin content in brain regions that influence the respiratory drive, such as the hypothalamus. Injection of natural progesterone leads to an increase in extracellular serotonin content in the hypothalamus [188], and mRNA levels of monoamine oxidase A, the enzyme involved in the catabolism of serotonin, decreases in the hypothalamus following the administration of natural progesterone [189].

In conclusion, progesteronergic systems facilitate breathing in healthy subjects by genomic and non-genomic mechanisms that include the modulation of neurotransmission systems, such as the serotoninergic systems. Finally, natural progesterone appears to influence breathing by its actions in the medullary and hypothalamic areas of the brain.

Because of the clinical potential of progesteronergic systems for inducing an increase in the respiratory drive, the question of their ability to induce beneficial effects in CHS patients was of particular interest.

4. RECENT CLINICAL AND ANIMAL DATA SUGGEST THAT GONANES ENHANCE VENTILATION IN CCHS UNLIKE NATURAL PROGESTERONE, PREGNANES, OR ESTRANES

4.1. CCHS is the most Highly Documented CHS and is Related to a Mutation of the *PHOX2B* Gene and Characterized by a Severe Diminished CO_2/H^+ Chemoreflex

CCHS is the subject of many active clinical and experimental studies, making it the most highly documented CHS. It is also called Ondine's curse syndrome in reference to "the story of a water sprite from European lore who cursed her unfaithful lover to lose all autonomic functions and therefore stop breathing when he fell asleep" [29]. It is a rare disorder, first described by Mellins and collaborators in 1970, with a very poor prognosis and high mortality during infancy. Children with CCHS typically present during the newborn period a documented failure of the autonomic control of breathing and various findings of autonomic nervous system dysfunction [190]. CCHS is diagnosed by hypoventilation linked to a severely diminished CO_2/H^+ chemoreflex (increase in central respiratory drive caused by elevation of CO_2 levels) in the absence of perceived respiratory discomfort and primary pulmonary, cardiac, and neuromuscular disorders or brain lesions that may account for the entire phenotype. In the most severe forms, mortality is high during the early postnatal period and hypoventilation is present during both sleep and wakefulness [31]. It occurs throughout the world, with an estimated incidence of 1 per 200,000 live births in France [30, 191, 192]. It is related to a heterozygous mutation in the PHOX2B gene [193]. The PHOX2B gene mutation consists of a polyalanine repeat expansion (from 4 to 13) in 90% of cases and missense, nonsense, or frameshift mutations in the remaining 10%. Introduction of the most frequent PHOX2B gene mutation in mice results in the dysfunction of PHOX2B-positive neurons of a medullary region called the retrotrapezoid/parafacial respiratory group (RTN/pFRG), a pivotal group for CO_2 chemoreception [16, 193-198]. The existence of the RTN/pFRG in humans has not been clearly established. However, based on immunohistochemical studies [199-201], CCHS is considered to mostly, if not completely, result from a dysfunction of the PHOX2B-positive and CO_2/H^+ sensing cells of the RTN/pFRG [31].

To date, no available pharmacological treatment exists for CCHS patients, which consequently depend on mechanical ventilation at least during sleep. This underlines the interest in developing pharmacological alternatives.

4.2. Recent Clinical and Animal Data Suggest that the Gonane Class of Progestins may be of Great Clinical Interest for Treating CCHS Patients

4.2.1. Recovery of CO_2 Chemosensitivity in CCHS Under Desogestrel Treatment

In the regular clinical follow-up of a CCHS patient from the French cohort [191], we were surprised to observe a respiratory response to CO_2 during a test using the rebreathing method [50, 202]. The patient was a 19-year-old woman carrying the five-alanine expansion mutation of the *PHOX2B* gene. She had been completely unresponsiveness to such stimulation since birth as illustrated in Fig. (**2A**), obtained less than three years before the observation of a respiratory response to CO_2. The patient exhibited a 2-3-fold increase in ventilation in two tests: breathing through a one-way valve in an open circuit of which the inspiratory arm was connected to a bag containing a 7% CO_2-93% O_2 gas mixture and two days later with the rebreathing method (Fig. **2A**). Extensive questioning of the patient allowed the physicians to suggest that the use of a progestin, desogestrel (75µg daily) as a contraceptive may have been involved in this beneficial effect. We tested this hypothesis, by assessing the ventilatory response of another patient, a 30-year-old woman carrying the six-alanine expansion mutation of the *PHOX2B* gene, to hypercapnia after the initiation of contraceptive treatment with desogestrel (75µg daily). The absence of ventilatory and sensory responses to hypercapnia by the rebreathing method had been documented several times for the second patient, as for the first, before the administration of desogestrel. Three weeks after the beginning of desogestrel treatment, she displayed a respiratory response to CO_2, with respiratory sensation and a marked anxiety that she had never experienced previously during such test. Given the known effect of natural progesterone on the central ventilatory drive, these observations led us to hypothesize that desogestrel, a progestin of the gonane group, was responsible for restoring the ventilatory response to CO_2, despite the absence of a known effect of natural progesterone or progestin from other molecular families in CCHS [46, 47]. However, shortly after this first study, another research group presented contradictory results. In this second clinical study, a unique patient, a 23-year-old woman carrying the seven-alanine expansion mutation of the *PHOX2B* gene, was deliberately treated for two weeks with 150 µg desogestrel

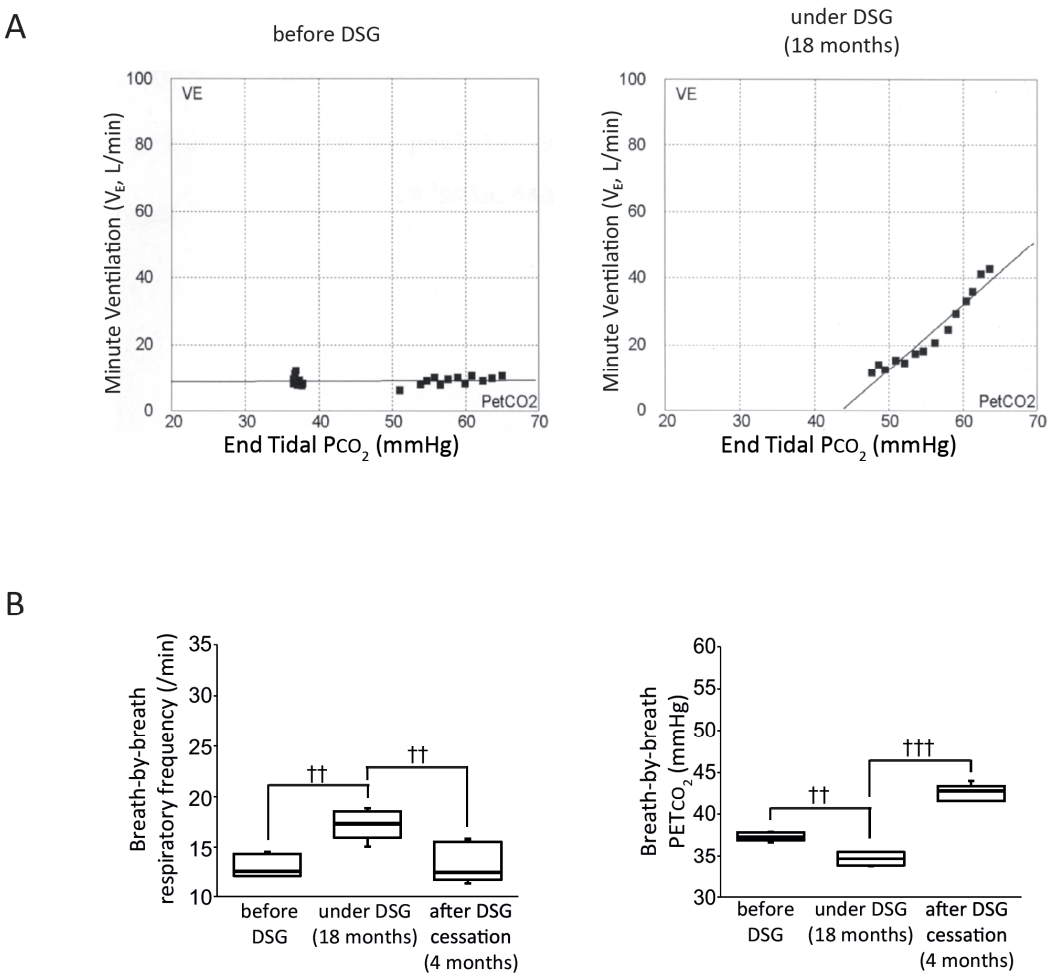

Fig. (2). Consequences of desogestrel exposure on the ventilation of a CCHS patient. (A) Ventilatory response of a CCHS patient during a CO_2 rebreathing test [50]. The left panel shows the change in ventilation (V_E) with the increase in end tidal CO_2 partial pressure assessed before desogestrel exposure. The right panel shows the same assessment after approximately 18 months of treatment with desogestrel. Reprinted from Respiratory Physiology & Neurobiology, 171(2), C Straus, H Trang, M-H Becquemin, P Touraine, T Similowski, Chemosensitivity recovery in Ondine's curse syndrome under treatment with desogestrel, 171-174, Copyright (2010), with permission from Elsevier. **(B)** Box plot showing the median breath-by-breath respiratory frequency and median breath-by breath end tidal CO_2 partial pressure of the CCHS patient at baseline [52]. † indicates a significant difference between before and after desogestrel treatment and during desogestrel exposure. One-way ANOVA – post hoc Bonferroni correction. $^{††}p < 0.01$, $^{†††}p < 0.001$. Abbreviation: desogestrel (DSG). Reprinted from Neuropharmacology, 107, F Joubert, A-S Perrin-Terrin, E Verkaeren, P Cardot, M-N Fiamma, A Frugière, I Rivals, T Similowski, C Straus, L Bodineau, Desogestrel enhances ventilation in ondine patients: Animal data involving serotoninergic systems, 339-350, Copyright (2016), with permission from Elsevier.

and 30 µg ethinyl estradiol daily and did not display a recovery of CO_2 chemosensitivity [203]. These contradictory findings may be due to the complex nature of the actions of progestins on breathing control. As previously discussed, natural progesterone, its metabolites, and therefore synthetic progestins, are known to possess a wide range of targets within the CNS (Fig. **1**) [103, 145, 147, 150, 161, 204, 205]. These contrary results may also be idiosyncratic. The action of desogestrel, or rather its metabolite 3-ketodesogestrel (etonogestrel), a synthetic progestin derived from testosterone and belonging to the gonane family [57, 205], may involve multiple pathways. Gonane progestins may exert their ventilatory effect, as natural progesterone and its derivatives (Fig. **1**), by acting on the hypothalamus or brainstem [182, 184] through both genomic [205] and non-genomic mechanisms [84, 132, 184, 206]. In such a scenario, the gonanes may be more potent than natural progesterone and other synthetic progestins, explaining why gonanes can have an effect on CCHS patients, whereas other drugs do not. Another possibility is that the gonanes exert a specific action, distinct from that of natural progesterone, its derivatives, or progestins other than gonane. In both cases, gonanes could activate or over-activate one or more central mechanisms controlling CO_2/H^+ chemosensitivity outside of the main chemosensitive central site, the RTN/pFRG, which is dysfunctional in CCHS due to the PHOX2B mutation.

The fortuitous observation of a recovery of CO_2 chemosensitivity in CCHS patients under desogestrel treatment

that does not seem to be systematical underlines the necessity to dissect the action mechanisms of this progestin.

4.2.2. The Desogestrel Metabolite Enhances the Respiratory Response to Acidosis in Presence of Supramedullary Areas

Extensive knowledge of the exact effect and modalities of action of desogestrel or its active metabolite, etonogestrel, on the central respiratory network is necessary for both evaluating the conditions under which these progestins could be used to treat CHS patients and determining the origin of the responsiveness of the CCHS patients to these drugs. These considerations led to studies on *ex vivo* preparations of CNS from rodents [207], making it possible to focus on the actions of progestin that target the CNS. *Ex vivo* preparations of CNS tissue from newborn rodents is a classical model used in central respiratory drive studies (Fig. **3**). These preparations are minimally composed of the medulla oblongata and spinal cord (MS preparations). They contain the neuronal groups of the medulla oblongata, essential for the elaboration of the respiratory rhythm *i.e.* the respiratory generators [4, 5], as well as other groups involved in the modulation of the respiratory rhythm, such as raphe nuclei [24, 208-212]. The rhythmic activity recorded at the level of the fourth ventral cervical root of the spinal cord is recognized as respiratory-like activity since the seminal study of Suzue (Fig. **3**) [213]. This has led researchers to use this preparation for many years for both pharmacological and CO_2/O_2 respiratory adaptation studies [207, 214-225]. Using *ex vivo* preparations containing encephalic regions more rostral than the medulla oblongata, diencephalon-brainstem-spinal cord preparations (DBS), permits the study of the influence of these rostral encephalic regions on the central respiratory drive [219, 226]. We assessed the effect of desogestrel/etonogestrel by comparing the respiratory-like activity of MS and DBS preparations subjected to a metabolic acidosis challenge in the presence or absence of etonogestrel [51]. Metabolic acidosis is an experimental situation that models hypercapnia [215, 227]. In accordance with the literature, metabolic acidosis induced an increase in the frequency of the respiratory-like activity by approximately 30% in the absence of progestin (Fig. **3**) [215, 227-229]. Etonogestrel exposure increased the respiratory response to metabolic acidosis of DBS but not MS preparations. This led us to propose that the gonane progestin may at least partially compensate for the neuronal deficit in CCHS patients through a mechanism involving encephalic regions rostral to the medulla oblongata. These observations suggest that CO_2/H^+-activated cells of supramedullary regions, such as those of the locus coeruleus [17, 230, 231], the periaqueductal gray matter [19, 21], and the caudal hypothalamus [20, 232], all reported to be progesterone-sensitive [233, 234], may be activated or over-activated by the gonane progestin. Further experiments are clearly necessary to identify the cell populations involved and characterize the etonogestrel-activated mechanisms at the cellular and molecular levels.

In addition to the facilitating effect of gonane progestins during a chemoreflex situation, based on the known influence of natural progesterone and its derivatives, the question of an effect of gonane progestins on the baseline ventilation was raised.

4.2.3. The Beneficial Effect of Desogestrel Extends Beyond $CO_2/H+$ Chemosensitivity in Baseline Ventilation

Given the effects of natural progesterone and other progesteronergic drugs on basal ventilation, it seemed potentially informative to analyze the effects of these gonane progestins outside of a CO_2/H^+ challenge to explore other effects on respiratory control. We thus carried out a retrospective analysis of the data recorded during baseline ventilation in the two CCHS patients who had a respiratory response to CO_2 under desogestrel [50]. The review of the dynamically collected data (before, during, and after desogestrel exposure) showed that the respiratory frequency was higher and $PETCO_2$ lower in the presence than absence of the progestin, whereas the tidal volume did not change (Fig. **2B**) [52]. This suggests that the ventilatory effect of desogestrel may extend beyond CO_2/H^+ chemosensitivity. We thus carried out a translational study on mice to dissect the action of etonogestrel on the baseline respiratory drive. First, we established that the facilitatory effect of etonogestrel on the respiratory frequency observed in humans was also present in mice. Using whole body plethysmography [235], we observed an increase in basal respiratory frequency in mice induced by etonogestrel at a similar proportion to that observed in humans, at a concentration near that of human exposure, given its bioavailability [236] (Fig. **4A**) [52]. Using mouse *ex vivo* CNS preparations that contained only the medulla oblongata and the spinal cord (MS preparations, Fig. **3**), we showed that the medulla oblongata is sufficient for the observed increase in respiratory frequency induced by etonogestrel (Fig. **4B**) [52]. Moreover, etonogestrel did not induce changes in the amplitude of the respiratory-like activity, similar to our observations in CCHS patients (increased respiratory frequency with no change of tidal volume). These observations suggest that the acceleration of the respiratory frequency by etonogestrel in CCHS patients, as well as in mice *in vivo*, may at least in part be mediated by medullary mechanisms. The RTN/pFRG, the medullary oscillator that is probably missing in CCHS patients because of the *PHOX2B* gene mutation [193, 196], did not seem to be involved in the accelerating effect of etonogestrel, as suggested by the absence of an increase in the number of cells positive for c-FOS, a marker of neuronal activity under etonogestrel exposure [52]. This histological observation in rodents is consistent with the increase in respiratory frequency observed in CCHS patient lacking *a priori* a functional RTN/pFRG.

Following the demonstration of the facilitating effect of gonane progestins on the respiratory frequency, the next step was to characterize the underlying mechanisms by searching for etonogestrel modulation of signaling involved in the functioning of the respiratory network.

4.2.4. The Desogestrel Metabolite Influences the Modulation of the Respiratory Drive Through the $GABA_A$ and NMDA Signaling in the Respiratory Network

We assumed that the action of etonogestrel depended, at least in part, on the interaction with membrane receptors, as reported for natural progesterone or its derivatives (Fig. **1**), because the acceleration of the respiratory frequency observed in mice *in vivo* and *ex vivo* was visible after a single acute application. We tested this hypothesis by using of

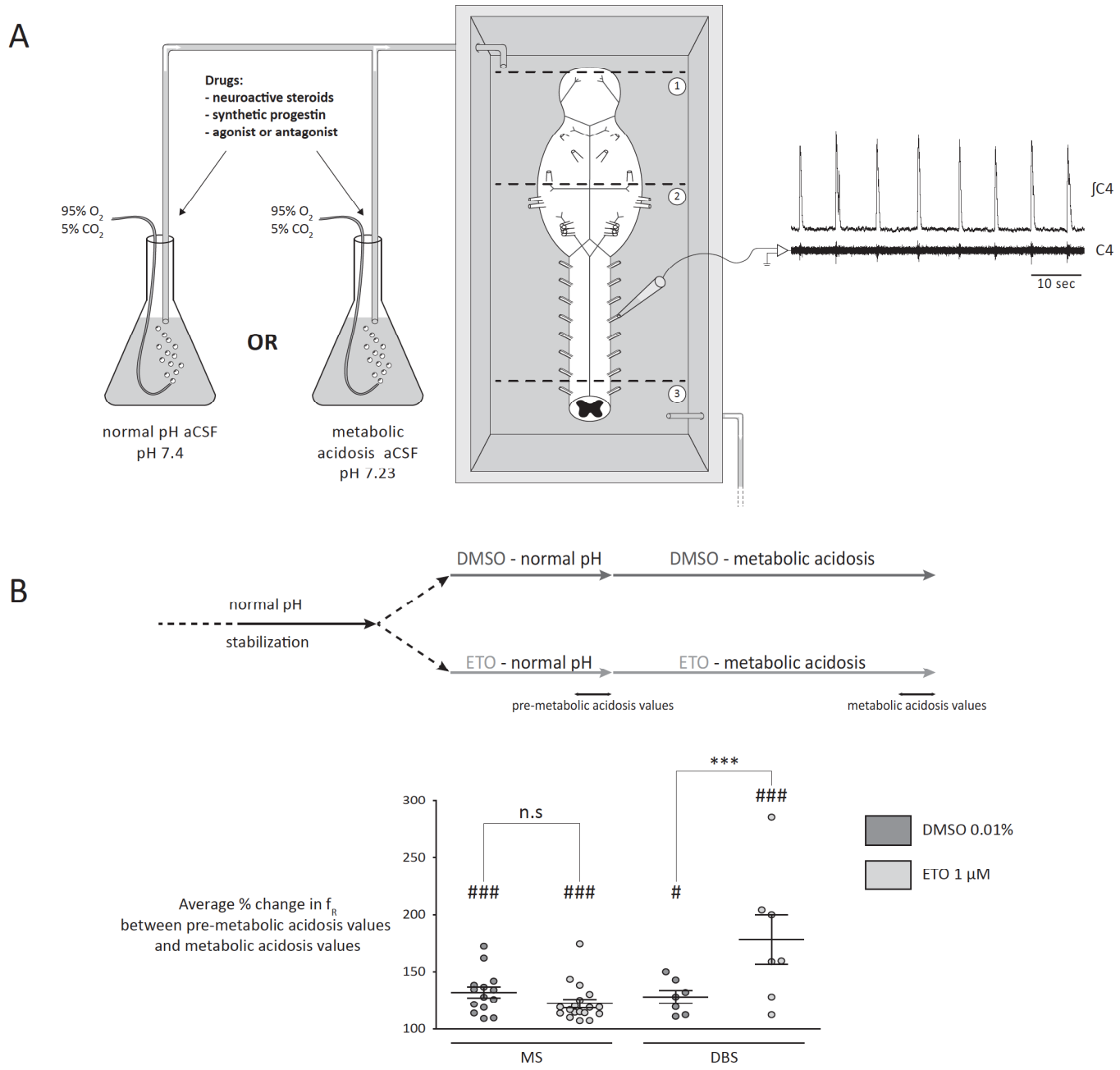

Fig. (3). ***Ex vivo*** **CNS preparations in an electrophysiological recording chamber and the effect of etonogestrel on respiratory frequency under conditions of metabolic acidosis on these preparations. (A)** Schematic representation of an *ex vivo* preparation of the CNS from a newborn rodent placed in an electrophysiological recording chamber. After the surgical procedure, *ex vivo* preparations were placed in a recording chamber and superfused either with artificial cerebrospinal fluid (aCSF) corresponding to normal pH (pH 7.4 aCSF: 129.0 mM NaCl, 3.35 mM KCl, 1.26 mM $CaCl_2$ $2H_2O$, 1.15 mM $MgCl_2$ $6H_2O$, 0.58 mM NaH_2PO_4 H_2O, 21.0 mM $NaHCO_3$, 30.0 mM D-glucose) or metabolic acidosis (pH 7.23 aCSF: 129.0 mM NaCl, 3.35 mM KCl, 1.26 mM $CaCl_2$ $2H_2O$, 1.15 mM $MgCl_2$ $6H_2O$, 0.58 mM NaH_2PO_4 H_2O, 15.0 mM $NaHCO_3$, 30.0 mM D-glucose), both saturated with O_2 and adjusted to pH by bubbling with 95% O_2 and 5% CO_2. Pharmacological agents can be added to aCSF according to the experimental conditions. The illustrated *ex vivo* preparation contains the medulla oblongata and supramedullary regions up to the diencephalon. Dotted lines show the levels of transection: the rostral extremity for diencephalon-brainstem-spinal cord (DBS) preparations (1) and medullary-spinal cord (MS) preparations (2) and the caudal extremity for both MS and DBS preparations (3). A typical electrophysiological respiratory-like activity recording at the level of ventral C4 and its integration is presented to the right. **(B)** Experimental protocol performed to test the effect of etonogestrel on CNS preparation from newborn rat under conditions of metabolic acidosis [51]. After a stabilization period (30 min superfused with normal pH aCSF without drug), preparations were pre-incubated 15 min either with etonogestrel (1 µM) or dimethyl sulfoxide (DMSO, 0.01%, the solvent for etonogestrel) *i.e* pre-metabolic acidosis period, before being superfused for 30 min with metabolic acidosis aCSF containing the same drugs *i.e.* metabolic acidosis period. Pre-metabolic acidosis

Fig. (3). contd….

and metabolic acidosis values were defined as the mean calculated over the last 5 min of the pre-metabolic acidosis and metabolic acidosis periods, respectively. The dot plot shows the percentage change in respiratory frequency (f_R) between respective pre-metabolic acidosis and metabolic acidosis values obtained in MS and DBS preparations exposed to DMSO (dark gray circles) or etonogestrel (light gray circles). All values are expressed as the mean ± SEM. Two-way ANOVA followed by a post hoc Bonferroni correction were performed. Differences were considered to significant for $p < 0.05$. # significant increase in mean f_R compared to control values; * significant difference between etonogestrel and DMSO exposures. # $p < 0.05$, ### $p < 0.001$, *** $p < 0.001$, n.s.: non-significant. Abbreviations: artificial cerebrospinal fluid (aCSF); integrated activity of the C4 ventral nerve root (∫C4); electrical activity of the C4 ventral nerve root (C4); dimethyl sulfoxide (DMSO); etonogestrel (ETO); respiratory frequency (f_R), medullary-spinal cord (MS); diencephalon-brainstem-spinal cord (DBS). Adapted from [51]. Reprinted from Neuroscience Letters, 567, C Loiseau, D Osinski, F Joubert, C Straus, T Similowski, L Bodineau, The progestin etonogestrel enhances the respiratory response to metabolic acidosis in newborn rats. Evidence for a mechanism involving supramedullary structures, 63-67, Copyright (2014), with permission from Elsevier.

ex vivo MS preparations to assess the influence of etonogestrel on the modulation of the respiratory-like frequency through the $GABA_A$ and NMDA signaling in the respiratory network, as both $GABA_A$ and NMDA receptors are involved in the modulation of the central respiratory drive [237-239] and are influenced by steroids [130, 132, 155]. First, blocking the $GABA_A$ receptors with bicuculline reduced the accelerating effect of etonogestrel at a low concentration (0.05 µM) and suppressed it at higher a concentration (2µM; Fig. **4C**) suggesting that part of the effect of etonogestrel may depend on the interaction of the progestin with $GABA_A$ receptors. These results are consistent with published data establishing steroid-dependent negative and/or positive allosteric modulation of $GABA_A$ receptor as indicated above [130, 206]. The differential effect of etonogestrel may be due to a concentration-dependent effect on several subunits of $GABA_A$ receptors. Indeed, the $GABA_A$ receptor-dependent response mediated by receptors containing $\alpha_{1/3}$ subunits is enhanced by low steroid concentrations, whereas receptors composed of $\alpha_{2/4/6}$ subunits require higher steroid concentrations [132]. The reduction or total suppression of the etonogestrel effect by bicuculline may depend on the overall efficiency of different types of $GABA_A$ receptors as $\alpha_{1/3}$ and $\alpha_{2/4}$ $GABA_A$ receptor subunits are both present in medullary respiratory areas [240, 241]. In addition, in the presence of etonogestrel, a $GABA_A$ receptor agonist, muscimol at IC_{50}, induced a greater decrease in respiratory frequency than in its absence, suggesting that the gonane progestin positively modulates $GABA_A$ receptor (Fig. **4D**). This was supported by the fact that gonanes such as etonogestrel are derived from testosterone (Fig. **1**), a positive modulator of $GABA_A$ receptor [130]. Concerning a possible interaction with the NMDA receptor, blocking of NMDA by the antagonist MK-801 completely suppressed the accelerating action of etonogestrel on respiratory frequency at low etonogestrel concentration, whereas the increase in respiratory frequency induced by NMDA was greater in the presence of etonogestrel than in its absence. Both observations suggest that etonogestrel influences the modulation of the respiratory-like frequency by NMDA signaling in the respiratory network.

The raised step was to investigate if the change of $GABA_A$ and NMDA signaling in the respiratory network influence a major central system of respiratory regulation known to be modulated by natural progesterone, the serotonergic system.

4.2.5. The Desogestrel Metabolite Increases the Baseline Respiratory Drive by a Medullary Serotoninergic Pathway

Taking into account several lines of evidence from the literature, it is plausible that the acceleration of the baseline respiratory drive by etonogestrel involves serotoninergic systems. Indeed, *i*) medullary serotoninergic neurons modulate the baseline central respiratory drive [208, 211, 242-245], *ii*) part of the acceleration of the respiratory drive by natural progesterone depends on an interaction with serotoninergic systems [39, 246], and *iii*) medullary serotoninergic neurons are thought to be influenced by GABA/$GABA_A$ and NMDA signaling [247-252]. Thus, we used a functional immunohistochemical approach to search for an eventual change of activity in the medullary raphe nuclei, raphe pallidus and raphe obscurus, two areas known to play a role in respiratory control [209, 212, 253]. Using c-FOS and dual c-FOS/serotonin immunohistochemical detection, we observed increased activity of cells in the caudal part of the raphe pallidus and raphe obscurus nuclei in the presence of etonogestrel (Fig. **5A**), with many of the c-FOS-positive cells also positive for serotonin (Fig. **5A**) [52]. In addition, blockade of the serotoninergic modulation of the respiratory drive by methysergide, a $5\text{-}HT_{1,2,7}$ receptor antagonist [225, 254-256], completely suppressed the accelerating effect of etonogestrel on the respiratory-like frequency in MS preparations (Fig. **5B**) [52]. These convergent results suggest the existence of a new interaction pathway between progesteronergic and serotoninergic systems, as the effects of steroids on the release of serotonin depends on supramedullary regions [246, 257]. The gonane-serotonin medullary interaction that affects the respiratory drive is likely specific to this progestin family.

CONCLUSION

In conclusion, among progesteronergic drugs, gonanes, such as desogestrel or its active metabolite etonogestrel, are of great potential interest in the context of CHS neurorespiratory pathologies. Gonane progestins can increase the respiratory drive at baseline. They also seem able to restore the respiratory response to CO_2 in CCHS patients who lack this chemosensitivity. Animal studies suggest that acceleration of the baseline respiratory drive by gonane progestins is related, at least in part, to a medullary serotoninergic pathway and that the recovery of the CO_2 chemoreflex depends on supramedullary action(s). The mechanisms likely involve the interaction of gonane with $GABA_A$ and NMDA membrane

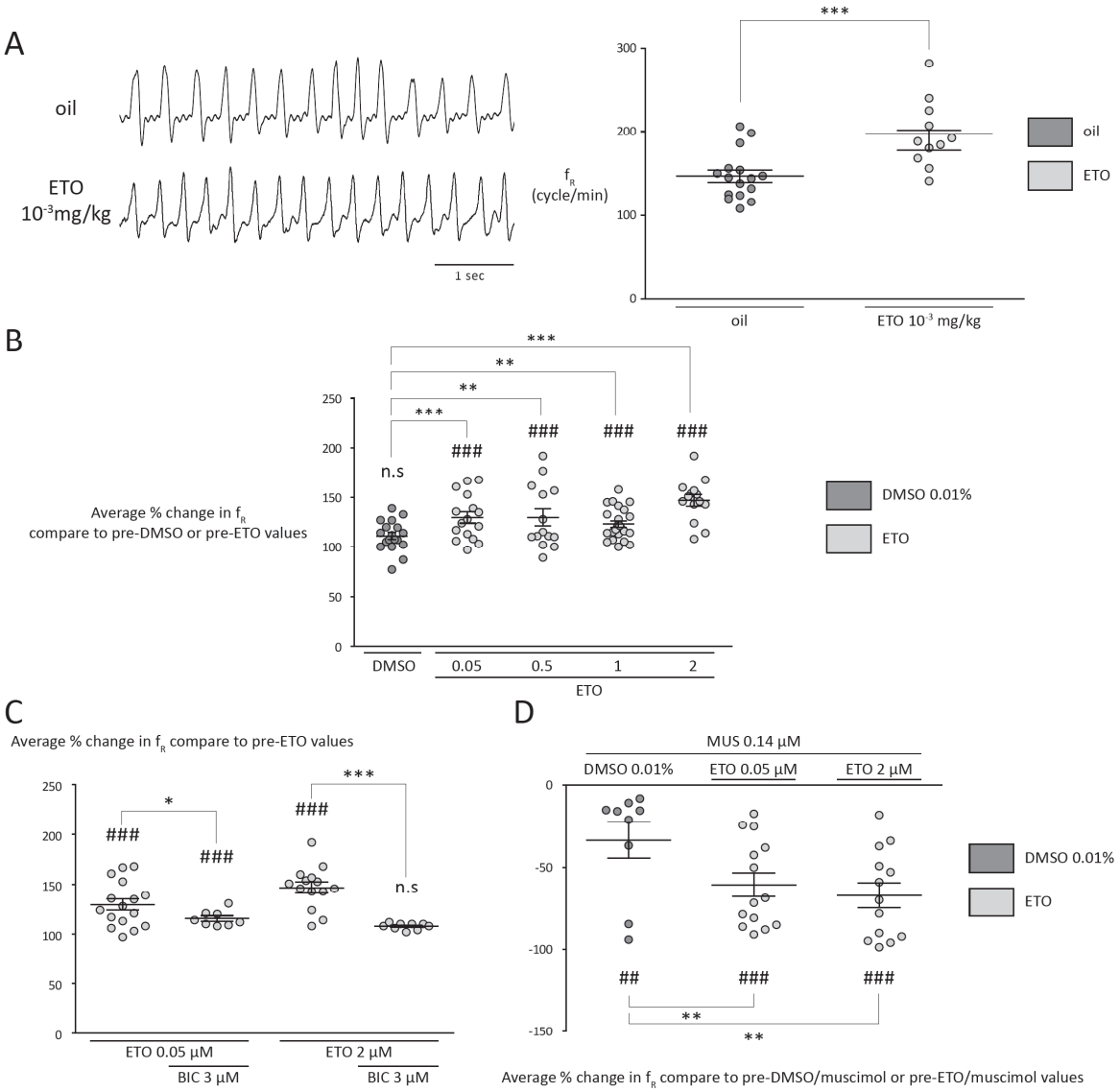

Fig. (4). Effect of etonogestrel on baseline respiratory frequency on *in vivo* newborn mice and *ex vivo* medullary-spinal cord preparations. Implication of $GABA_A$ receptors. (A) Effect of etonogestrel on baseline respiratory frequency (f_R) on *in vivo* newborn mice. Newborn mice received *per os* either etonogestrel (ETO; 10^{-3} mg/kg; concentration equivalent to that of the human exposure) dissolved in oil or oil alone. The f_R was recorded by whole body plethysmography. Traces illustrate ventilation after oil or etonogestrel exposure. The dotplot shows the baseline f_R obtained after oil (dark gray circles) or etonogestrel (light gray circles) exposure. **(B)** Effect of etonogestrel on f_R of *ex vivo* medullary-spinal cord preparations. Preparations were exposed to etonogestrel (0.05, 0.5, 1, or 2 µM) or dimethyl sulfoxide (DMSO, 0.01%, etonogestrel solvent). The respiratory-like activity was recorded at the level of the fourth cervical ventral root (Fig. **3**). The dot plot shows the percentage change of the mean f_R during DMSO (dark gray circles) or etonogestrel (light gray circles) exposure. **(C)** Effect of etonogestrel under conditions of $GABA_A$ receptor blockade on *ex vivo* medullary-spinal cord preparations. Preparations were pre-incubated with bicuculline ($GABA_A$ receptor antagonist) before etonogestrel exposure. The dotplot shows the percentage change of the f_R during etonogestrel exposure (0.05 µM and 2 µM) in the absence and presence of 3 µM bicuculline (a concentration previously shown to be sufficient to block the effect of GABA on $GABA_A$ receptor -mediated regulation of respiration [206]). **(D)** Effect of etonogestrel on $GABA_A$ receptor modulation of *ex vivo* medullary-spinal cord preparations. Preparations were first exposed to etonogestrel (0.05 µM and 2 µM) or DMSO (0.01%) and then DMSO/muscimol (0.14 µM; EC_{50}, as determined in [52]) or etonogestrel/muscimol (0.14 µM). The dotplot shows the percentage change of the f_R during DMSO/muscimol (dark gray circles) or etonogestrel /muscimol (light gray circles) exposure. All values are expressed as the mean ± SEM. The student t-test or two-way ANOVA, followed by post hoc Bonferroni correction were performed as appropriate. Differences were considered to be significant for $p < 0.05$. # significant increase in mean f_R relative to pre-DMSO and pre- etonogestrel values as appropriate; * significant difference between etonogestrel and DMSO or oil exposures or between the absence and presence of bicuculline. ## $p < 0.01$, ### $p < 0.001$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s.: non-significant. Abbreviations: bicuculline (BIC); dimethyl sulfoxide (DMSO); etonogestrel (ETO); respiratory frequency (f_R); muscimol (MUS). Adapted from [52]. Reprinted from Neuropharmacology, 107, F Joubert, A-S Perrin-Terrin, E Verkaeren, P Cardot, M-N Fiamma, A Frugière, I Rivals, T Similowski, C Straus, L Bodineau, Desogestrel enhances ventilation in ondine patients: Animal data involving serotoninergic systems, 339-350, Copyright (2016), with permission from Elsevier.

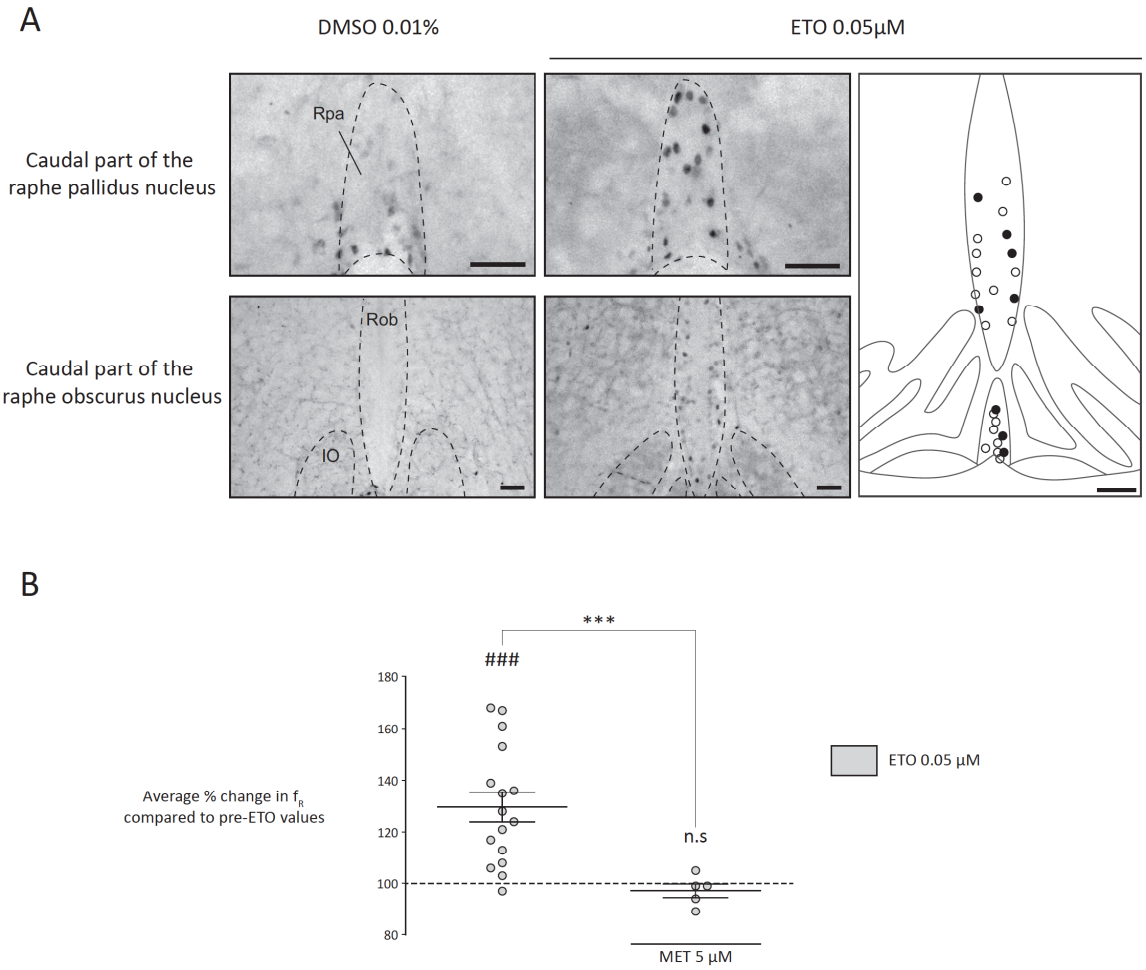

Fig. (5). The effect of etonogestrel on respiration implies the medullary serotoninergic systems. (A) Effect of etonogestrel on *c-fos* expression in medullary respiratory structures containing serotoninergic neurons. On the left, gray photomicrographs illustrate c-FOS immunoreactivity in the caudal parts of the pallidus and obscurus raphe nuclei after dimethyl sulfoxide (DMSO) or etonogestrel exposure. Scale bar: 100 µm. On the right, photomicrographs illustrate the serotoninergic character of c-FOS-immunoreactive cells in these two structures. Scale bars: 10 µm. **(B)** Effect of etonogestrel under conditions of blockade of serotoninergic respiratory influence on *ex vivo* medullary-spinal cord preparations. Preparations were first exposed to methysergide (a 5-$HT_{1/2/7}$ receptor antagonist; 5 µM, the lowest concentration that completely antagonizes the effect of 5-HT, as determined in [52]) and then etonogestrel (0.05 µM). The scatter plot shows the percentage change of the f_R during etonogestrel exposure in the absence or presence of methysergide. All values are expressed as the mean ± SEM. The Kruskal-Wallis test was performed. Differences were considered to be significant for $p < 0.05$. # significant increase in mean f_R relative to pre-ETO values; * significant difference between the presence and absence of methysergide. ### $p < 0.001$, *** $p < 0.001$, n.s.: non-significant. Abbreviations: etonogestrel (ETO); inferior olives (IO); respiratory frequency (f_R); methysergide (MET). Adapted from [52]. Reprinted from Neuropharmacology, 107, F Joubert, A-S Perrin-Terrin, E Verkaeren, P Cardot, M-N Fiamma, A Frugière, I Rivals, T Similowski, C Straus, L Bodineau, Desogestrel enhances ventilation in ondine patients: Animal data involving serotoninergic systems, 339-350, Copyright (2016), with permission from Elsevier.

receptors leading to a change of the modulation of the respiratory-like frequency through the $GABA_A$ and NMDA signaling in the respiratory network. Further experiments are necessary to completely understand the central actions of these progestins, in particular regarding the complexity of the action of progesteronergic drugs in the context of patients who respond [50] or not [203] to the gonane.

LIST OF ABBREVIATIONS

5-HT	=	5-Hydroxytryptamine
aCSF	=	Artificial cerebrospinal fluid
AF	=	Activation function
AR	=	Nuclear androgen receptor
BIC	=	Bicuculline
C4	=	Electrical activity of the C4 ventral nerve root
CCHS	=	Congenital central alveolar hypoventilation syndrome
CHO	=	Chinese hamster ovary
CHS	=	Central alveolar hypoventilation syndromes

CNS	=	Central nervous system
CO_2	=	Carbon dioxide
DBD	=	DNA binding domain
DBS	=	Diencephalon-brainstem-spinal cord
DMSO	=	Dimethyl sulfoxide
DSG	=	Desogestrel
EC_{50}	=	Half maximal effective concentration
ETO	=	Etonogestrel
f_R	=	Respiratory frequency
$GABA_A$	=	Type A gamma-aminobutyric acid receptor
GPCR	=	G-protein-coupled receptor
GR	=	Nuclear glucocorticoid receptor
h	=	Nuclear location signal
HEK	=	Human embryonic kidney
IF	=	Inhibition factor
IO	=	Inferior olives
LBD	=	Ligand binding domain
MET	=	Methysergide
MK-801	=	Dizocilpine
mPR	=	Membrane progesterone receptor
MR	=	Nuclear mineralocorticoid receptor
mRNA	=	Messenger ribonucleic acid
MS	=	Medullary-spinal cord
MUS	=	Muscimol
NMDA	=	N-methyl-D-aspartate
NTD	=	N-terminal domain
O_2	=	Dioxygen
P450c17	=	Steroid 17-alpha-hydroxylase/17,20 lyase precursor
$PaCO_2$	=	Partial pressure of carbon dioxide in arterial blood
PaO_2	=	Partial pressure of dioxygen in arterial blood
$PETCO_2$	=	Partial pressure of end-tidal carbon dioxide
PHOX2B	=	Paired-like homeobox 2B
pFRG	=	Parafacial respiratory group
PGRMC1	=	Progesterone receptor membrane component 1
PR	=	Nuclear progesterone receptor
PRE	=	Progesterone responsive element
RTN	=	Retrotrapezoid nucleus
RU486	=	Mifepristone
∫C4	=	Integrated activity of the C4 ventral nerve root
THDOC	=	3α5α tetrahydrodeoxycorticosterone
UTR	=	Untranslated region
V_E	=	Minute ventilation

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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Camille Loiseau performed animal experiments, analyzed data, discussed the results and their significance, generated figures and wrote part of the manuscript. Florence Cayetanot wrote part of the manuscript. Fanny Joubert performed animal experiments, analyzed data, discussed the results and their significance and generated figures. Anne-Sophie Perrin-Terrin performed animal experiments, analyzed data and discussed the results and their significance. Philippe Cardot, Marie-Noëlle Fiamma and Alain Frugiere discussed part of the presented results and their significance. Christian Straus performed clinical observations, analyzed data and discussed the results and their significance. Laurence Bodineau designed and supervised all animal experiments, obtained funding, shaped, and interpreted the data, discussed the results and their significance, and wrote the manuscript.

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