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Electrophysiological effects of various neuroactive steroids on the $GABA_A$ receptor in pituitary melanotrope cells

Frank Le Foll, Estelle Louiset, Hélène Castel, Hubert Vaudry, Lionel Cazin *

European Institute for Peptide Research (IFRMP No. 23), Laboratory of Cellular and Molecular Neuroendocrinology, INSERM U413, UA CNRS, University of Rouen, 76821 Mont-Saint-Aignan, France

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Abstract

The action of steroids on the bioelectrical response to γ-aminobutyric acid (GABA) has never been studied in pituitary cells. In the present study, we have thus investigated the effects of a series of neuroactive steroids on the GABA-activated current in frog melanotrope cells in primary culture, using the patch-clamp technique in the whole-cell configuration. Bath perfusion of 3α-isomers of pregnanolone or tetrahydrodeoxycorticosterone (1 μM) significantly enhanced the current evoked by short pulses of GABA (3 μM) and accelerated its desensitization. In contrast, the 3β-isomers (30 μM) had no effect on the GABA-activated current. Addition to the bath solution of dehydroepiandrosterone or dehydroepiandrosterone sulfate (10 μM) inhibited the GABA-activated current without modifying its kinetics while pregnenolone sulfate (10 μM) both inhibited the GABA-activated current and accelerated its decay rate. The effects of pregnane steroids were not impaired by the central-type benzodiazepine receptor antagonist flumazenil (10 μM). In conclusion, the present study reveals that neuroactive steroids may exert multiple modulatory activities on the $GABA_A$ receptor borne by melanotrope cells. The effect of steroids on the current evoked by GABA is rapid, reversible, stereospecific and not mediated through the benzodiazepine binding site of the $GABA_A$ receptor. © 1997 Elsevier Science B.V.

Keywords: GABA (γ-aminobutyric acid); Pregnanolone; Tetrahydrodeoxycorticosterone; Dehydroepiandrosterone; Pregnenolone sulfate; Neuroactive steroid; Pituitary melanotrope cell; Patch-clamp

1. Introduction

The neurotransmitter γ-aminobutyric acid (GABA) regulates the activity of several adenohypophysial cell types. In the rat pituitary, GABA, acting through $GABA_A$ receptors, stimulates the secretion of luteinizing hormone (Anderson and Mitchell, 1986a; Virmani et al., 1990), growth hormone and adrenocorticotropin (Anderson and Mitchell, 1986a) and exerts a dual effect, successively stimulatory and inhibitory, on prolactin secretion (Anderson and Mitchell, 1986b). In the rat or porcine pars intermedia, activation of $GABA_A$ receptors enhances the secretion of α-melanocyte-stimulating hormone (α-MSH) (Demeneix et al., 1984, 1986). In contrast, in the pars intermedia of the toad *Xenopus laevis*, $GABA_A$ receptor agonists inhibit α-MSH release (Verburg-van Kemenade et al., 1987; Jenks et al., 1993). In the frog *Rana ridibunda*, activation of $GABA_A$-receptors induces a biphasic response, i.e., a rapid stimulation followed by a sustained inhibition of α-MSH secretion (Adjeroud et al., 1986; Desrues et al., 1995).

The activity of the $GABA_A$ receptor can be regulated by various pharmacological agents including benzodiazepines, barbiturates, ethanol, as well as Zn^{2+} ions (Aguayo and Alarcon, 1993). The $GABA_A$ receptor is also the target of several neuroactive steroids (Harrison and Simmonds, 1984; Majewska et al., 1986). Up to now, the effect of steroids on the GABA-activated chloride current has been investigated in chromaffin cells (Callachan et al., 1987; Peters et al., 1988), neurons (Demirgören et al., 1991; Spivak, 1994) and in human embryonic kidney 293 (HEK293) cells transfected with various $GABA_A$ receptor subunits (Puia et al., 1990; Zhu et al., 1996). Although GABA and steroid hormones are major regulators of pituitary hormone secretion, little is known about the modulation of $GABA_A$ receptors by neuroactive steroids in hypophysial cells (Vincens et al., 1989). In particular, the

* Corresponding author. Tel.: (33-2) 3514-6675; Fax: (33-2) 3514-6946.

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electrophysiological effects of steroids on GABA-activated currents have never been investigated in pituitary cells. The frog pars intermedia which is composed of a single population of endocrine cells (Benyamina et al., 1986) expressing $GABA_A$ receptors (Louiset et al., 1990, 1994), provides a valuable model in which to investigate the modulation of the electrical response to GABA by neuroactive steroids.

Herein, we have studied the effects of various neuroactive steroids on the GABA-activated currents in cultured frog melanotrope cells by using the patch-clamp technique in the whole-cell configuration.

2. Materials and methods

2.1. Reagents and test substances

Protease type IX, collagenase type IA, Leibowitz L-15 medium, antibiotics, GABA, 5α-pregnan-3α-ol-20-one (5α3α preg), 5β-pregnan-3α-ol-20-one (5β3α preg), 5α-pregnan-3β-ol-20-one (5α3β preg) and 5β-pregnan-3β-ol-20-one (5β3β preg), 5α-pregnan-3α,21-diol-20-one (5α3α THDOC), 5β-pregnan-3α,21-diol-20-one (5β3α THDOC) and 5α-pregnan-3β,21-diol-20-one (5α3β THDOC), dehydroepiandrosterone, dehydroepiandrosterone sulfate, pregnenolone sulfate, pentobarbital and *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) were purchased from Sigma (St. Louis, MO, USA). Fetal calf serum was supplied from Biosys (Compiègne, France). Plastic culture dishes were obtained from C.M.L. (Nemours, France). The benzodiazepine receptor ligands flumazenil and clonazepam were a generous gift from Dr. Heafly (Hoffmann-La Roche, Basel, Switzerland).

2.2. Cell culture

Frog melanotrope cells were isolated and cultured as previously described (Louiset et al., 1988). Briefly, eight neurointermediate lobes from European green frog (*Rana ridibunda*) pituitaries were dissected and rinsed in Leibovitz L-15 medium (diluted 2:3 to adjust to amphibian osmolality) supplemented with a kanamycine and antibiotic–antimycotic solution (1% v/v). The tissues were enzymatically dissociated at 22°C in the same medium containing 0.15% protease and 0.15% collagenase for 15 min. The digested tissue was disaggregated by gentle aspiration through a siliconized Pasteur pipette. Dispersed cells were centrifuged ($60 \times g$, 5 min) and resuspended four times in the culture medium supplemented with 10% fetal calf serum. The cells were plated in the same medium at a density of 10 000 cells per 35 mm plastic culture dish and kept 5–10 days at 22°C in a humidified atmosphere.

2.3. Patch-clamp recordings

Electrophysiological studies were conducted at room temperature (20–22°C) using the standard patch-clamp technique (Hamill et al., 1981) in the whole-cell configuration and the voltage-clamp mode. The culture medium was replaced by a bathing solution containing 112 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$ and 15 mM HEPES (pH 7.4) and the cells were allowed to adapt to the new medium for 30 min. The recording electrodes were fabricated from 1.5 mm (outer diameter) soft glass tubes on a two-step vertical pipette puller (List-Medical, L/M-3P-A, Darmstadt, Germany). The solution used to fill the patch pipette had the following composition: 100 mM potassium glutamate, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, 10 mM EGTA and 2 mM ATP (pH 7.4). The resistance of the electrodes filled with this solution was 3 to 5 MΩ. Recordings were made with an Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA, USA), filtered at 1 kHz (−3 dB, four-pole, low-pass Bessel filter) and digitized at a sampling rate of 0.27 kHz using a Digidata 1200 interface (Axon Instruments) and the pClamp 6.0.2 acquisition software (Axon Instruments). The liquid junction potential was measured as described (Neher, 1992) and a correction of −13 mV was applied before the establishment of the whole-cell configuration.

2.4. Drug application

Stock solutions of steroids were prepared in ethanol and diluted prior to use in the bathing solution so that the final concentration of ethanol was always lower than 0.1%. Previous experiments have shown that this concentration of ethanol does not affect the electrophysiological response to GABA (Louiset et al., 1992). During the recordings, the cells were continuously superfused with the bathing solution at a flow rate of 3 ml/min. All test substances except GABA were added to the superfusion fluid. GABA was applied by expelling a small volume (1 μl) from a calibrated micropipette just upstream to the cell and close to the cell surface.

2.5. Data analysis

Peak current amplitudes were determined by the analysis software. Quantitative data are expressed as mean ± S.E.M. Statistical comparisons were performed using a one-way ANOVA. The percentage of modulation of the GABA-activated current was calculated as $I_{test}/I_{control} \times 100$, where $I_{control}$ and I_{test} are the peak amplitudes of the GABA-activated current in the absence and presence of the test substance, respectively. When measured in the presence of flumazenil, the effect of modulators was calculated as $I_{test+FLZ}/I_{test} \times 100$, where I_{test} and $I_{test+FLZ}$ are the peak amplitudes of the GABA-activated current in the presence of the test substance alone and in combination with flumazenil, respectively. Fittings of the dose–response relationships were performed using the logistic equation:

$$I = I_{max} \times [\text{steroid}]^{n_H} / \left(EC_{50}^{n_H} + [\text{steroid}]^{n_H}\right)$$

where I_{max} is the maximal current amplitude, expressed as the percentage of its control value, [steroid] is the concentration of steroid applied, EC_{50} the concentration of steroid that produces 50% of I_{max} and n_H the Hill coefficient.

3. Results

The effects of steroids on the GABA-activated current (I_{GABA}) were studied in 129 cultured melanotrope cells. The transmembrane current was recorded by using a low chloride solution in the patch pipette at a holding potential of 0 mV. At the beginning of each set of recordings, the cells were exposed to three successive pulses of GABA at 2 min intervals. Only cells responding to GABA without any run-down were selected. To study potentiating effects of steroids, GABA was applied at a concentration (3 μM) known to induce half-maximal current. Inhibitory actions of steroids were investigated on currents evoked by 10 μM GABA, a concentration at which the maximal response was obtained (Louiset et al., 1994).

3.1. Amplitude and time-course of the GABA-activated current

A brief microejection of GABA in the vicinity of the cells generated a transient outward current. The current

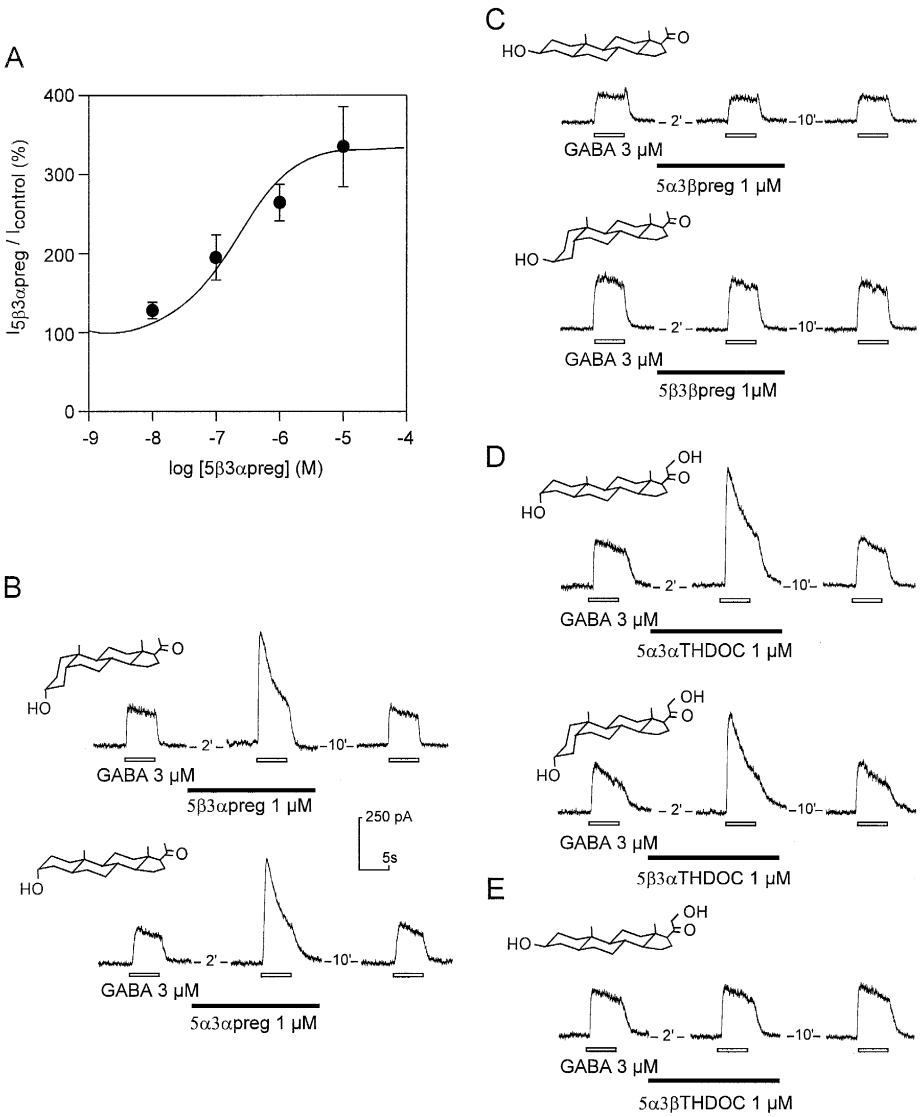

Fig. 1. Effects of pregnane steroids on the GABA-activated current in cultured frog melanotrope cells. (A) Concentration-dependent potentiation of the GABA-activated current by the 3α-isomer of pregnanolone, 5β3α preg. The current evoked by 3 μM GABA was expressed as the percentage of its control value. Each point represents mean ± S.E.M. ($n = 7$ to 14). The curve corresponds to the best fit of the experimental points to the dose–response equation specified in Section 2. (B–E) Effects of various pregnane steroids on the GABA-activated current. The current traces were recorded before (left), during (middle) and after (right) bath perfusion (filled bars) of 1 μM of the 3α-isomers of pregnanolone (B), 3β-isomers of pregnanolone (C), 3α-isomers of THDOC (D) or a 3β-isomer of THDOC (E). GABA (3 μM) was pressure-ejected in the proximity of the cells for 5 s (open bars). The chemical structure of each steroid is represented above the current traces. Holding potential was 0 mV.

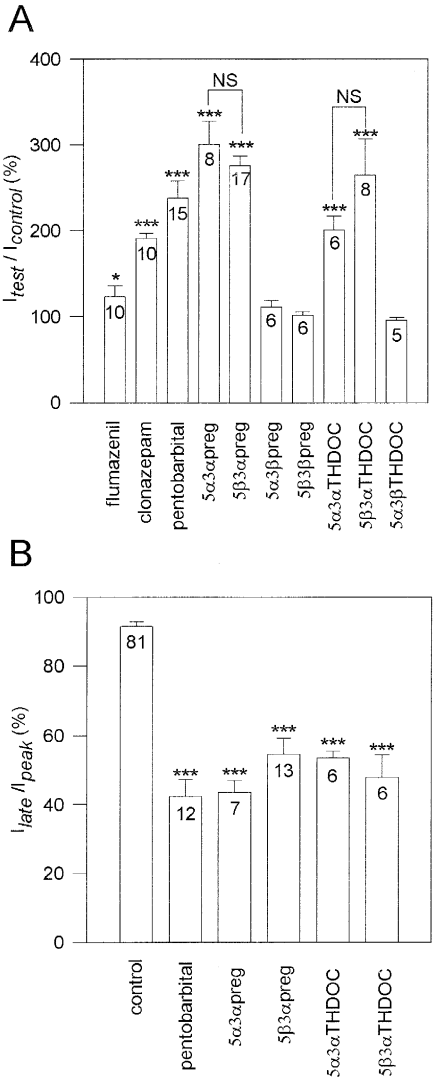

Fig. 2. Effects of benzodiazepines, pentobarbital or pregnane steroids on the GABA-activated current in cultured frog melanotrope cells. (A) Bar graph representing the amplitude of the GABA (3 μM)-activated current, expressed as the percentage of its control value, in the presence of 10 μM flumazenil, 1 μM clonazepam, 100 μM pentobarbital, 1 μM 5α3αpreg, 1 μM 5β3αpreg, 30 μM 5α3βpreg, 30 μM 5β3βpreg, 1 μM 5α3αTHDOC, 1 μM 5β3αTHDOC or 30 μM 5α3βTHDOC. (B) Bar graph representing the effects on the relative current desensitization of 100 μM pentobarbital, 1 μM 5α3αpreg, 1 μM 5β3αpreg, 1 μM 5α3αTHDOC or 1 μM 5β3αTHDOC. Relative current desensitization corresponds to the ratio of the current measured at the end of the GABA pulse (I_{late}) to the peak current (I_{peak}). In (A) and (B), the columns represent mean values ± SEM and the numbers are the numbers of cells tested (* $P < 0.05$, *** $P < 0.001$, NS non-significant difference, one-way ANOVA).

elicited by a 5 s pulse of 3 μM GABA displayed a mean peak amplitude of 107 ± 5 pA ($n = 81$) and slowly decayed during the exposure to GABA. The late current, measured at the end of the GABA application, was 91 ± 1% of the peak current. When used at a concentration of 10 μM, GABA was applied only during 2 s, in order to avoid tachyphylaxis. Under these conditions, the current reached

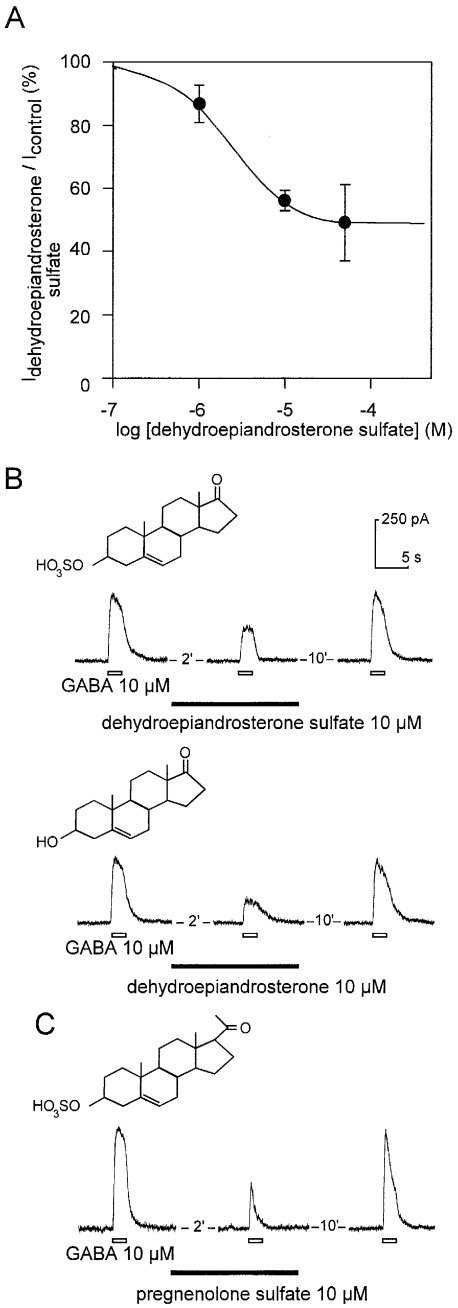

Fig. 3. Effects of dehydroepiandrosterone sulfate, dehydroepiandrosterone or pregnenolone sulfate on the GABA-activated current in cultured frog melanotrope cells. (A) Concentration-dependent inhibition of the GABA-activated current by dehydroepiandrosterone sulfate. The current evoked by 10 μM GABA was expressed as the percentage of its control value. Each point represents mean ± S.E.M. ($n = 6$). The curve corresponds to the best fit of the experimental points to the dose–response equation specified in Section 2. (B–D) Effects of 10 μM dehydroepiandrosterone sulfate (B), dehydroepiandrosterone (C) and pregnenolone sulfate (D) on the GABA-activated current. The current traces were recorded before (left), during (middle) and after (right) bath perfusion of steroids (filled bars). GABA (10 μM) was pressure-ejected in the proximity of the cell for 2 s (open bars). The chemical structure of each steroid is represented above the current traces. Holding potential was 0 mV.

a mean peak amplitude of 552 ± 19 pA ($n = 78$) and the late current was 88.2 ± 0.9% of the peak current.

3.2. Effects of pregnane steroids on the GABA-activated current

The effects of pregnane steroids were investigated in 59 melanotrope cells. Incubation of the cells with pregnanolone (5β3α preg), at concentrations ranging between 0.01 and 10 μM, produced a dose-dependent and reversible increase of the GABA-activated current (Fig. 1A,B). The best fit of the dose–response curve was obtained with $EC_{50} = 0.17$ μM, $I_{max} = 327\%$ and $n_H = 1.0$ ($n = 7$ to 14). Similarly, superfusion with the 3α-isomer of pregnanolone 5α3α preg enhanced I_{GABA} (Fig. 1B). The potentiating effect of both 3α-isomers on I_{GABA} was marked by an acceleration of the current desensitization. In contrast, the 3β-isomers (5α3β preg or 5β3β preg), did not affect I_{GABA} (Fig. 1C).

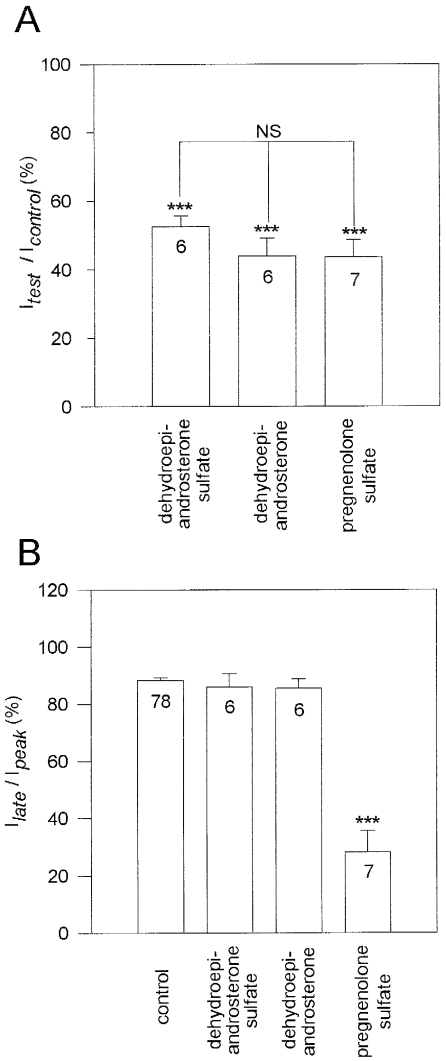

Fig. 4. Effects of dehydroepiandrosterone sulfate, dehydroepiandrosterone or pregnenolone sulfate on the amplitude and desensitization of the GABA-activated current in cultured frog melanotrope cells. (A) Bar graph representing the amplitude of the GABA (10 μM)-activated current, expressed as the percentage of its control value, in the presence of 10 μM dehydroepiandrosterone sulfate, dehydroepiandrosterone or pregnenolone sulfate. (B) Bar graph representing the effects on the relative current desensitization of 10 μM dehydroepiandrosterone sulfate, dehydroepiandrosterone or pregnenolone sulfate. Relative current desensitization corresponds to the ratio of the current measured at the end of the GABA pulse (I_{late}) to the peak current (I_{peak}). In (A) and (B), the columns represent mean values ±S.E.M. and the numbers are the numbers of cells tested (*** $P < 0.001$, NS non-significant difference, one-way ANOVA).

In order to test whether the presence of an additional hydroxyl group had an influence on the potentiating effect of pregnane steroids, cells were submitted to bath perfusion with tetrahydrodeoxycorticosterone isomers. In the presence of 5α3α THDOC or 5β3α THDOC, the GABA-activated current was markedly potentiated and the current desensitization was accelerated (Fig. 1D). As the 3β-isomers of pregnanolone, 5α3β THDOC did not modify I_{GABA} (Fig. 1E). None of the tested steroids had any effect on the basal membrane current of the cells.

The effects of pregnane steroids on the amplitude of I_{GABA} were compared to those of benzodiazepine receptor ligands and barbiturates (Fig. 2A). The benzodiazepine receptor antagonist flumazenil, at a high concentration (10 μM), slightly increased I_{GABA} ($P < 0.05$), whereas the benzodiazepine receptor agonist clonazepam (1 μM) and the barbiturate pentobarbital (100 μM) markedly augmented the current amplitude ($P < 0.001$). The potentiating effects of 5α3α preg and 5β3α preg (1 μM) did not significantly differ each other ($P > 0.1$). Similarly, the potentiating effects of the 5α3α THDOC and 5β3α THDOC (1 μM) did not differ either ($P > 0.1$). In contrast, the 3β-isomers of pregnanolone or THDOC, even at a high concentration (30 μM), did not modify I_{GABA} ($P > 0.1$).

The effects of barbiturates and active pregnane steroids on current desensitization are presented in Fig. 2B. Incubation of the cells with pentobarbital significantly enhanced the desensitization process ($P < 0.001$). In very much the same way, all active steroids including the 3α-isomers of prenanolone and THDOC caused a pronounced reduction (50–40%) of the relative amplitude of the late current ($P < 0.001$).

3.3. Effects of dehydroepiandrosterone sulfate, dehydroepiandrosterone and pregnenolone sulfate on the GABA-activated current

The effects of dehydroepiandrosterone sulfate, dehydroepiandrosterone and pregnenolone sulfate on the GABA-activated current were investigated in a total of 40 melanotrope cells. Superfusion of dehydroepiandrosterone sulfate at concentrations ranging between 1 and 50 μM inhibited I_{GABA} in a dose-dependent manner, the fit parameters of the dose–response curve yielding $EC_{50} = 2.36$

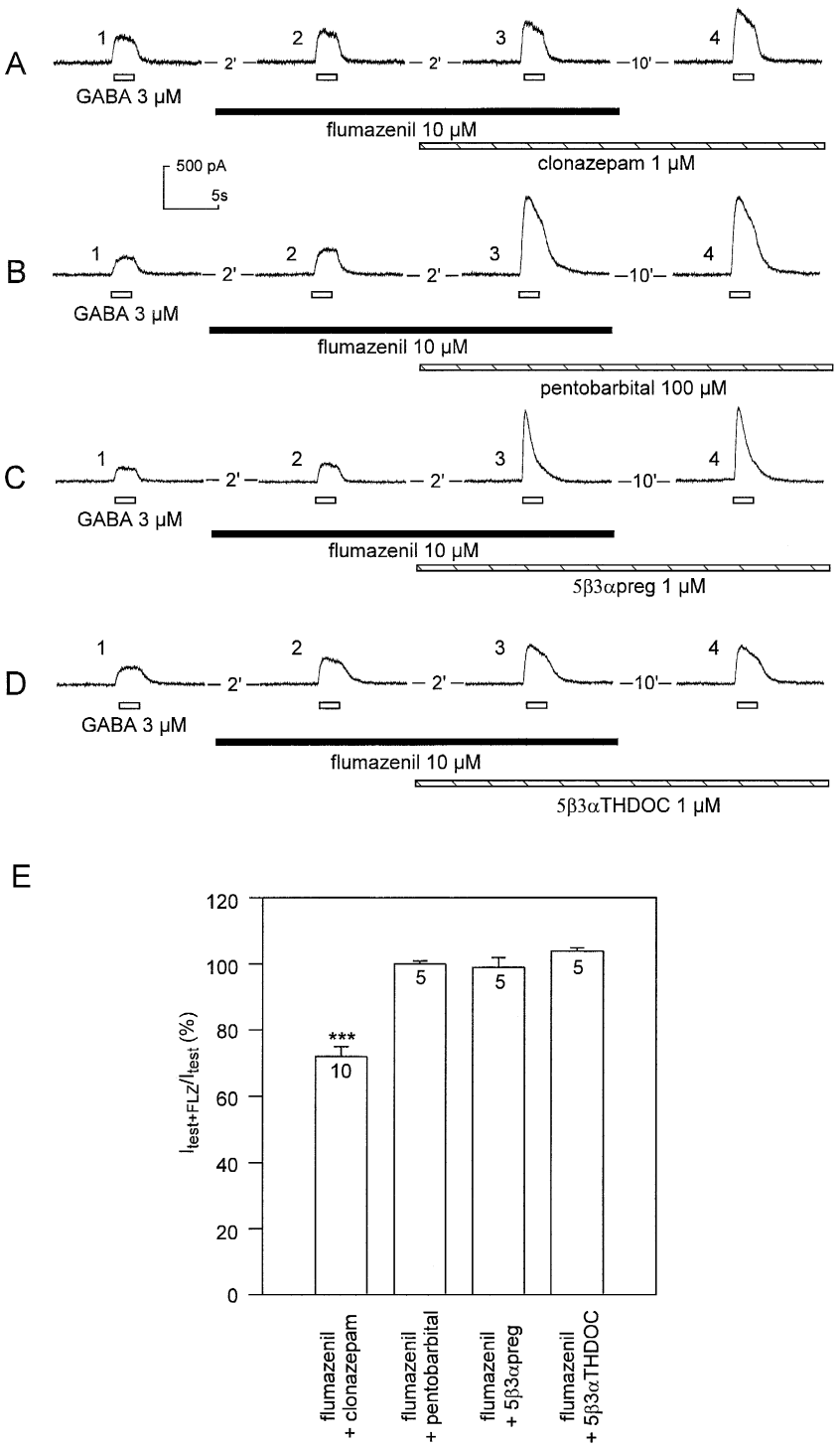

Fig. 5. Effects of flumazenil on the modulation of the GABA-activated current by clonazepam, pentobarbital or pregnane steroids in cultured frog melanotrope cells. (A–D) The current traces were recorded before (1), during (2, 3) and after (4) 10 μM flumazenil administration (filled bars). Bath perfusion of 1 μM clonazepam (A), 100 μM pentobarbital (B), 1 μM 5β3αpreg (C) or 1 μM 5β3αTHDOC (D) was performed as indicated by the hatched bars below the current traces (3, 4). GABA (3 μM) was pressure-ejected in the proximity of the cell for 2 s (open bars). (E) Bar graph representing the amplitude of the GABA (3 μM)-activated current in the presence of a combination of 10 μM flumazenil and 1 μM clonazepam, 1 μM pentobarbital, 1 μM 5β3αpreg or 1 μM 5β3αTHDOC. The amplitude of the GABA-activated current (3) was expressed as the percentage of the control value measured in the presence of the potentiator alone (4). The columns represent mean values ±S.E.M. and the numbers are the numbers of cells tested (*** $P < 0.001$, one-way ANOVA).

μM, $I_{max} = 51\%$ and $n_H = 1.2$ ($n = 6$, Fig. 3A,B). Both dehydroepiandrosterone sulfate and dehydroepiandrosterone attenuated the GABA (10 μM)-activated current but had no effect on the time-course of the residual current (Fig. 3B,C). The effects of dehydroepiandrosterone sulfate and dehydroepiandrosterone reversed after washout. Bath perfusion with 10 μM pregnenolone sulfate produced a reversible inhibition of the current evoked by 10 μM GABA pulses accompanied by a dramatic increase of the current desensitization, which persisted after extensive washout (Fig. 3D).

Quantification of the data revealed that dehydroepiandrosterone sulfate, dehydroepiandrosterone and pregnenolone sulfate all induced a significant inhibition of the GABA-activated current ($P < 0.001$, Fig. 4A). Dehydroepiandrosterone and dehydroepiandrosterone sulfate had no effect on the late current while pregnenolone sulfate significantly enhanced the current decay ($P < 0.001$, Fig. 4B).

3.4. Site of action of pentobarbital and pregnane steroids on the $GABA_A$ receptor

A series of experiments was conducted in order to investigate whether pentobarbital and pregnane steroids exert their effects through an interaction with the benzodiazepine-binding site of the $GABA_A$ receptor. The central-type benzodiazepine receptor agonist clonazepam potentiated the GABA-activated current. The central-type benzodiazepine receptor antagonist flumazenil significantly attenuated the potentiating effect of clonazepam on I_{GABA} ($P < 0.001$, Fig. 5A,E). In contrast, flumazenil did not modify the potentiating effect of pentobarbital (100 μM), $5\beta3\alpha$preg (1 μM) or $5\beta3\alpha$THDOC (1 μM) on the GABA-activated current (Fig. 5B–E).

4. Discussion

Previous studies have demonstrated the existence of $GABA_A$ receptors in the frog melanotrope cells (Adjeroud et al., 1986; Tonon et al., 1989). In particular, the response to GABA has been shown to be mimicked by the $GABA_A$ receptor agonist muscimol, totally blocked by the chloride channel blocker picrotoxin and potentiated by the central-type benzodiazepine agonist clonazepam (Louiset et al., 1990, 1992). In this respect, the pharmacological profile of the $GABA_A$ receptors expressed in these cells is similar to that of mammalian neurones (Akaike et al., 1986; Kumamoto and Murata, 1995). We now provide the first evidence that neuroactive steroids exert a dual action on the GABA-activated current in an endocrine cell type.

Our findings indicate that pregnane steroids enhanced the GABA-activated current in a dose-dependent manner in frog melanotrope cells. The pregnane steroids structure-activity relationships revealed that α-position of the substituting C3-hydroxyle is essential for potentiation. In contrast, more dramatic modifications in the steroid backbone structure, like the presence (THDOC isomers) or absence (pregnanolone isomers) of an additional hydroxyle on the C21 carbon, as well as the A-ring twist from the trans 5α- to the *cis*-fused 5β-pregnane form had little if any influence on the effect of the steroids. As a consequence, contrary to the nicotinic acetylcholine receptor (Sunshine and Mcnamee, 1994), the $GABA_A$ receptor does not appear to be modulated by steroids through any nonspecific modification of the lipid membrane composition or fluidity. The stringent structure-function relationships suggest a mechanism of action involving a specific molecular binding site for the pregnane steroids on the $GABA_A$ receptor.

The increase of I_{GABA} caused by the neuroactive steroids $5\alpha3\alpha$preg, $5\beta3\alpha$preg, $5\alpha3\alpha$THDOC and $5\beta3\alpha$THDOC was typically accompanied by an acceleration of the current desensitization. A similar phenomenon has already been reported in frog melanotrope cells for central-type benzodiazepine receptor agonists (Louiset et al., 1994). Since the acceleration of the current decay is associated with an increase of the current intensity, the rise of the ionic flow through the chloride channel might account for the current desensitization. The mechanism responsible for such a desensitization could be an ion-block process similar to that reported for calcium currents (Eckert and Tillotson, 1981). A transient decrease of the chloride driving force due to a local intracellular accumulation of chloride could also participate to the fading of the current (Huguenard and Alger, 1986). Such a phenomenon is likely to occur within the 5 s GABA applications, since simplified calculations have demonstrated that time constant of ion diffusion between pipette and intracellular solutions is about 4 s (Marty and Neher, 1995). Alternatively, the current decay could be ascribed to non-conducting desensitized $GABA_A$ receptor-channel states caused by the presence of neuroactive steroids.

The present data clearly establish that, in frog melanotrope cells, dehydroepiandrosterone sulfate dose-dependently inhibited the GABA-activated current. In addition, our study indicates that at the concentration of 10 μM, the effects of dehydroepiandrosterone sulfate or dehydroepiandrosterone were identical, suggesting that the sulfate group is not crucial for the action of these steroids. In this respect, the effects of dehydroepiandrosterone and dehydroepiandrosterone sulfate on the melanotrope cells differ from that described in rat neurons in which the desulfated form of the steroid appeared to be a less potent inhibitor of I_{GABA} (Demirgören et al., 1991). Moreover, our study shows that the inhibition of the GABA-activated current by both forms of dehydroepiandrosterone was never associated with any change in the current time-course. Again, this observation contrasts with the marked desensitization of the GABA-activated current detected in rat neurons in the presence of dehydroepiandrosterone sulfate (Demirgören et al., 1991; Spivak, 1994). The discrepancies

between frog pituitary cells and rat neurons can be ascribed to variations in the subunit compositions of the $GABA_A$ receptors, leading to distinct gating properties of the chloride channel (Gingrich et al., 1995).

Contrary to the effect of dehydroepiandrosterone (or dehydroepiandrosterone sulfate), the inhibition of the GABA-activated current provoked by pregnenolone sulfate was accompanied by a pronounced and durable current desensitization. Since pregnenolone sulfate caused a decrease of the chloride flux induced by GABA, it appears unlikely that the observed current desensitization was chloride-dependent. Thus, the current desensitization occurring in the presence of pregnenolone sulfate may rather result from conformational changes turning the $GABA_A$ receptor in a less conducting state. Altogether, our results suggest that the inhibitory effects of dehydroepiandrosterone (or dehydroepiandrosterone sulfate) and pregnenolone sulfate on the GABA-activated current are mediated through distinct mechanisms.

At last, we observed that the effects of pentobarbital and pregnane steroids on I_{GABA} were not impaired by flumazenil which attenuated the clonazepam-induced potentiation of I_{GABA}. Similar findings in rat cuneate neurons with alphaxalone (Harrison and Simmonds, 1984) and bovine adrenochromaffin cells with pregnanedione (Callachan et al., 1987) have previously been reported. The fact that the effects of barbiturates and pregnane steroids were not sensitive to a central-type benzodiazepine receptor antagonist strongly supports the view that these two classes of molecules and benzodiazepines act on distinct sites.

The present study has demonstrated that neuroactive steroids modulate the electrophysiological response to GABA at the pituitary level. The melanotrope cells appear to be a useful pharmacological model for studying the mode of action of steroids on the $GABA_A$ receptor. In these cells, neuroactive steroids exert a dual modulatory effect, i.e., potentiation or inhibition, on the GABA-activated current depending on the steroid structure. The stringent stereoselectivity of the potentiators and the differential effects of the inhibitors on the current desensitization suggest the existence of specific sites and mechanisms of action for steroids on the $GABA_A$ receptor. It is concluded that neuroactive steroids share the same signalling route than GABA to exert a very fine bidirectional tuning of a fast synaptic input of pituitary melanotrope cells.

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