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The effects of inhibitors of GABAergic transmission and stress on brain and plasma allopregnanolone concentrations

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1 This study was undertaken to investigate the relationship between a reduction in brain $GABA_A$ receptor function and the cerebro-cortical content of 3α-hydroxy-5α-pregnan-20 one (allopregnanolone, AP), a potent endogenous positive modulator of γ-aminobutyric acid (GABA) action at $GABA_A$ receptors, with anticonflict and anticonvulsant effects in rodents.

2 An acute depletion of the cerebral content of GABA or an attenuation of $GABA_A$ receptor-mediated transmission by systemic injections of isoniazid (375 mg kg^{-1}, s.c.) or FG 7142 (15 mg kg^{-1}, i.p.) induced a transient increase in the cerebro-cortical and plasma concentrations of AP in handling-habituated (not stressed) rats.

3 Two stress paradigms, handling in naive rats and mild foot shock in handling-habituated rats, that reduce central GABAergic tone mimicked the effects of isoniazid and FG 7142 on cortical AP content; foot shock in handling-habituated rats, but not handling in naive animals, also increased plasma AP. Isoniazid, FG 7142, and foot shock also each increased the concentrations of the AP precursors, pregnenolone and progesterone, in both brain and plasma of handling-habituated rats, whereas handling in naive rats increased the concentrations of these steroids only in brain.

4 Pretreatment of handling-habituated rats with the anxiolytic β-carboline derivative abecarnil, a positive allosteric modulator of $GABA_A$ receptors, which *per se* failed to affect the AP concentration in brain or plasma, prevented the increase in brain and plasma AP induced by foot shock or isoniazid.

5 In adrenalectomized and castrated rats foot shock or isoniazid failed to increase AP both in brain cortex and plasma.

6 These observations indicate that inhibition of GABAergic transmission, induced by foot shock or pharmacological manipulations, results in an increase in the concentrations of AP in brain and plasma, possibly via a modulation of hypothalamic-pituitary-adrenal (HPA) axis.

7 Given that AP enhances $GABA_A$ receptor function with high efficacy and potency, an increase in brain AP concentration may be important in the fine tuning of the GABA-mediated inhibitory transmission in the central nervous system.

Keywords: GABAergic transmission; allopregnanolone; isoniazid; FG 7142; abecarnil; stress; hypothalamic-pituitary-adrenal (HPA) axis; neurosteroids

Introduction

The neurosteroid 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, AP) is produced in the brain (Le Goascogne *et al*., 1987; Corpèchot *et al*., 1993; Mathur *et al*., 1993; Cheney *et al*., 1995) and shares with other natural and synthetic 3α-hydroxy steroids the ability to modulate the type A receptor of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in brain (for review see Majewska, 1992; Lambert *et al*., 1995). AP binds stereoselectively and with high affinity to a distinct steroid recognition site on the $GABA_A$ receptor complex, and thereby potentiates the response of the receptor-associated Cl^-conductance to GABA (Majewska *et al*., 1986; Peters *et al*., 1988; Puia *et al*., 1990; 1993; McCawley *et al*., 1995).

The interaction between AP and the $GABA_A$ receptor complex appears to underlie the pharmacological actions of AP. Thus, AP administered systemically or intracerebroventricularly elicits anxiolytic, anticonvulsant and neuroendocrine effects similar to those produced by other positive allosteric modulators of $GABA_A$ receptors such as benzodiazepines and barbiturates (Mendelson *et al*., 1987; Belelli *et al*., 1989; Bitran *et al*., 1991; Guo *et al*., 1995; Lambert *et al*., 1995; Concas *et al*., 1996). Similarly, anxiolytic effects are observed when the content of endogenous AP in brain is increased pharmacologically by systemic administration of its precursors (pregnenolone and progesterone) (Bitran *et al*., 1993; 1995; Romeo *et al*., 1993). Moreover, the anticonvulsant action of AP is enchanced when GABAergic transmission is reduced, as would be expected for an amplifier of GABA action and as has been demonstrated for ligands of central benzodiazepine receptors (Concas *et al*., 1996).

In rodents, exposure to various short-term stressors alters brain $GABA_A$ receptor function (Biggio *et al*., 1984; 1990; Havoundjian *et al*., 1986; Concas *et al*., 1988; Drugan *et al*., 1989; Serra *et al*., 1990; Sanna *et al*., 1992) and induces pro-conflict behaviour (Corda & Biggio, 1986). Acute stress (forced swim stress, CO_2 inhalation) also increases the amount of endogenous AP in rat brain (Purdy *et al*., 1991; Barbaccia *et al*., 1994; 1996b; Biggio *et al*., 1996) with a time course that better correlates with the recovery of $GABA_A$ receptor function, as assessed both biochemically and behaviourally, than with the early reduction in $GABA_A$ receptor function elicited by acute stress (Barbaccia *et al*., 1996b; Biggio *et al*., 1996). The increase in brain AP content after acute stress has therefore been interpreted as an adaptive mechanism that may counteract the decrease in GABAergic function.

Since acute stress elicits changes in $GABA_A$ receptor function that take place earlier than those in brain AP con-

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tent (Corda & Biggio, 1986; Barbaccia *et al*., 1996b), in this paper we have evaluated whether there is a relationship between the strength of $GABA_A$ receptor-mediated synaptic transmission and the brain content of AP. Experiments have been performed in which the time course of brain cortical AP changes have been analysed after exposure to acute stress paradigms, including handling in naive rats and foot shock in handling-habituated animals, that reduce $GABA_A$ receptor function (Biggio *et al*., 1984; 1990; Concas *et al*., 1988) or after administration of drugs that either inhibit GABAergic transmission, including the GABA synthesis inhibitor isoniazid (Horton, 1980) and the anxiogenic β-carboline derivative FG 7142, a negative allosteric modulator of $GABA_A$ receptors (Ninan *et al*., 1982; Corda *et al*., 1983; Dorow *et al*., 1983), or potentiate $GABA_A$ receptor function such as the anxiolytic β-carboline derivative abecarnil (Stephens *et al*., 1990; Stephens, 1993). Moreover, in order to locate the source of the brain cortical AP that is increased by foot shock, isoniazid and FG 7142 we have carried out experiments in which adrenalectomized/orchiectomized rats were subjected to these treatments.

Methods

Animals

Male Sprague-Dawley CD rats (Charles River, Como, Italy) with body masses of 200 to 250 g were housed under standard laboratory conditions with a 12 h light, 12 h dark cycle, a constant temperature of $22 \pm 2^{\circ}C$, and water and food *ad libitum*. Adrenalectomy/orchiectomy was performed under chloral hydrate anaesthesia (400 mg kg^{-1}, i.p.) and the rats received isotonic saline as drinking water for 21 days, until the day of death. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Handling and foot shock

Foot shock consisted of a series of electrical impulses delivered in individual boxes with floors made of brass rods, 2 cm apart. Shocks (0.2 mA for 500 ms) were delivered every second over a period of 5 min. The stress associated with killing was minimized by habituating the rats to the manoeuvres that precede killing for four consecutive days (Biggio *et al*., 1984; 1990; Concas *et al*., 1988); they were picked up from their cages, held for 1 min in the foot shock box, and either introduced in the microwave holder (for brain steroid measurements) or placed with their head under the blade of a guillotine (for plasma steroid measurements). After each session, that was repeated twice a day for 4 days until approximately 80% of the animals assumed their final position in the microwave holder or under the guillotine without opposing resistance, the rats were returned to their house cages. Rats subjected to pharmacological treatments were similarly habituated to the injection procedure.

Extraction and assay of AP and corticosterone

Animals were killed at the indicated times either by guillotine (for plasma steroid measurements) or by focused microwave irradiation (70 W cm^{-2} for 4 s) to the head (for brain steroid measurement). This latter procedure results in a virtually instantaneous inactivation of brain enzymes, thus minimizing *postmortem* steroid metabolism. Brains were rapidly (<1 min) removed from the skull, and the cerebral cortices were dissected and frozen at $-20^{\circ}C$ until steroid extraction. AP was extracted and purified as previously described (Barbaccia *et al*., 1996b). Briefly, AP present in cerebral cortical homogenates (400 mg of tissue protein in 4 ml of phosphate-buffered saline (pH 7.0)) was extracted three times with ethyl acetate and the combined organic phases were dried under vacuum. The residue was dissolved in 5 ml of *n*-hexane and applied to Seppak silica cartridges (Waters), and components were eluted with *n*-hexane and 2-propanol (7:3, vol:vol). AP was further purified by high-performance liquid chromatography (h.p.l.c.) on a 5-μm Lichrosorb-diol column (250 by 4 mm) (Merck) with a discontinuous gradient of 2-propanol (0 to 30%) in *n*-hexane. The recovery (70 to 80%) of AP through the extraction and purification procedures was monitored by adding trace amounts (4000 to 6000 c.p.m; specific activity, 50 Ci $mmol^{-1}$) of tritiated standard to the brain homogenate.

Protein concentration was measured by the method of Lowry *et al*., (1951).

Blood was collected from the trunk of rats killed by guillotine into heparinized tubes and centrifuged at 900 *g* for 20 min at room temperature; the plasma was frozen until assayed for steroids. Steroids were extracted from plasma (1 ml) three times with 3 volumes of ethyl acetate and assayed in portions of the extract corresponding to 10 and 0.1% of the original plasma volume for AP and corticosterone, respectively. AP and corticosterone were quantitated by radioimmunoassay of duplicate samples as previously described (Purdy *et al*., 1990; Barbaccia *et al*., 1994; 1996b). In random samples AP immunoreactivity was identified as authentic AP by gas chromatographic/mass spectrometric (GC/MS) analysis following derivatization with hetafluorobutyryc anhydride. The GC/MS system consisted of a Hewlett Packard (Palo Alto, CA, U.S.A.) model 5890, series II, and a model 5971A MSD. The chromatographic column was a Supelco SPB1 (30×0.25 mm; 0.25 μm film thickness). The mass spectrometry detector was operating in selected ion monitoring mode focused on the ions 514 (molecular ion) and 496 (most abundant ion).

Materials

[9,11,12-^{3}H(N)]-AP (50 Ci $mmol^{-1}$) and [1,2,6,7,-^{3}H(N)]-corticosterone (88 Ci $mmol^{-1}$) were obtained from Dupont Biotechnology Systems (Milan, Italy); abecarnil and FG 7142 (β-carboline-3carboxylic acid ethylestermethylamide) were from Schering (Berlin, Germany); and corticosterone, AP, and isoniazid were from Sigma (Milan, Italy). Rabbit or sheep antiserum to AP was generated and characterized as previously described (Purdy *et al*., 1990). Antiserum to corticosterone was obtained from ICN (Costa Mesa, CA). All other reagents and organic solvents (h.p.l.c. grade) were of the best available quality from commercial sources.

Statistical analysis

Data are presented as means $\pm$ s.e.means and were compared by analysis of variance followed by Scheffés test. A P value of <0.05 was considered statistically significant.

Results

Effect of acute stress on the amount of AP in the cerebral cortex

Handling (consisting of picking up the rats from their cages and either introducing them in the microwave holder or placing their head under the guillotine blade) of naive rats immediately before killing increased the concentration of AP and its precursors pregnenolone and progesterone in the cerebral cortex when compared with the value measured in handling-habituated rats (Table 1). Exposure to foot shock for 5 min also increased the cortical AP content in handling-habituated rats in a time-dependent manner (Figure 1a). The increase in cortical AP was detected (+71%) immediately after the foot shock session ended, peaked (+425%) after 30 min, and persisted for at least 60 min, returning to control values after 120 min.

Table 1 Effect of handling on brain cortical and plasma neuroactive steroid concentrations in naive (N) and handling-habituated (H-H) rats

	Pregnenolone		*Progesterone*		*AP*		*Corticosterone*
	Cortex (ng g^{-1} protein)	*Plasma* (ng ml^{-1})	*Cortex* (ng g^{-1} protein)	*Plasma* (ng ml^{-1})	*Cortex* (ng g^{-1} protein)	*Plasma* (ng ml^{-1})	*Plasma* (ng ml^{-1})
N	128±10**	1.6±0.3	70±9.0*	1.3±0.30	15.3±2.0*	0.86±0.11	70±20
H-H	77±8.0	1.4±0.2	40±7.0	1.3±0.25	8.6±1.2	0.72±0.12	85±18

Naive rats were handled only once immediately before death (i.e. rats were picked up from their cages and killed either introducing them in the microwave holder or placing their head under the guillotine blade for brain and plasma steroid measurements, respectively). Handling-habituated rats were habituated to the manoeuvres that precede killing (for details see Methods section) twice daily for 4 consecutive days. Each number represents the mean±s.e.mean of 8 individual determinations, each run in duplicate. *$P<0.05$, **$P<0.01$ when compared to the respective values in handling-habituated rats.

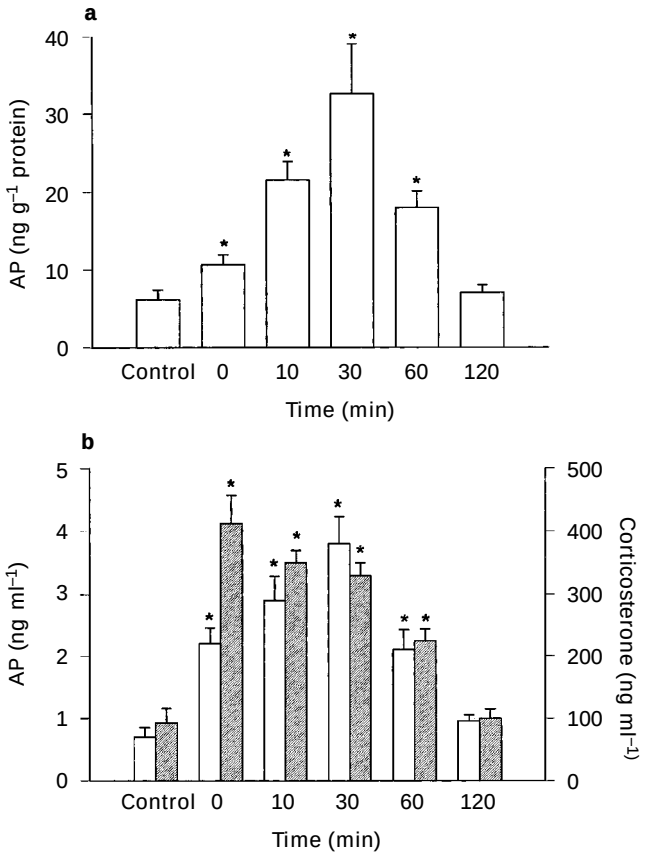

Figure 1 Time course of the changes in (a) cerebrocortical AP concentration and (b) plasma AP (open columns) and corticosterone (hatched columns) levels induced by foot shock. Handling-habituated rats were subjected to foot shock for 5 min and killed either immediately (time 0) or at the indicated times thereafter by focused microwave irradiation to the head (a) or by guillotine (b). Data are means±s.e.mean of 8 to 10 rats. *$P<0.05$, vs respective control (handling-habituated rats not subject to foot shock).

Effect of pharmacological inhibition of GABAergic transmission on AP concentration in the cerebral cortex

Subcutaneous administration of the GABA depletor isoniazid (375 mg kg^{-1}), an inhibitor of glutamic acid decarboxylase (Horton, 1980), increased the AP concentration in the cerebral cortex of handling-habituated rats in a time-dependent manner (Figure 2a). Consistent with the time course of GABA depletion induced by isoniazid (Horton, 1980), the increase in cortical AP (+460) was maximal 40 min after the administration of the drug and vanished by 120 min after drug injection. Since the GABA depletion caused by isoniazid may alter both $GABA_A$ and $GABA_B$ receptor mediated events, we also evaluated the effect of treatment with a selective negative allosteric modulator of $GABA_A$ receptor, the anxiogenic β-carboline derivative FG 7142 (Ninan *et al*., 1982; Corda *et al*., 1983; Dorow *et al*., 1983). Similar to foot shock and isoniazid, FG 7142 (15 mg kg^{-1}) increased (+300%) the brain cortical AP content 30 min post-injection and this effect vanished by 120 min (Figure 2a), consistent with the time course of its $GABA_A$ receptor-mediated proconvulsant and anxiogenic action (Corda *et al*., 1983).

Effect of stress and inhibition of GABAergic transmission on the concentration of AP and corticosterone in plasma

While handling in naive rats failed to alter the plasma concentration of AP, pregnenolone, progesterone and corticosterone, when compared to handling-habituated rats (Table 1), foot shock, isoniazid, and FG 7142 increased the plasma concentration of AP in handling-habituated rats with a time course similar to that observed in brain (Figures 1b and 2b), with maximal increases of +442 (30 min), +152 (40 min), and +85% (30 min), respectively. These treatments also increased the plasma concentrations of corticosterone (Figures 1b and 2b).

The kinetics of plasma corticosterone increase following foot shock in handling-habituated rats differed quite markedly from those of AP. While AP was maximally increased 30 min following foot shock, corticosterone was already at its maximal level immediately after the foot shock session and declined thereafter being significantly lower at time 30 (418±30 time 0; 324±20 time 30, $n=8$, $P<0.05$) (Figure 1b). Isoniazid and FG 7142 increased plasma corticosterone less dramatically and its changes were parallel to those of AP (Figure 2b).

Effect of abecarnil on the increase in cortical AP induced by foot shock or isoniazid

In handling-habituated rats pretreated with the positive allosteric modulator of $GABA_A$ receptor and anxiolytic β-carboline derivative abecarnil (0.3 mg kg^{-1}, i.p.) 30 min before foot shock, the latter treatment failed to increase the concentration of AP in the cerebral cortex (Figure 3) or in plasma (not shown). This dose of abecarnil, which was previously shown to exert maximal anxiolytic and anticonvulsant action (Stephens, 1993), *per se* had no effect on the cortical and plasma concentrations of AP in control handling-habituated rats. Abecarnil pretreatment also prevented the isoniazid-induced increase in the brain cortical and plasma concentration of AP in handling-habituated rats (Figure 3).

Effect of foot shock, isoniazid and FG 7142 on the AP concentration in the cerebral cortex and plasma of Adx/Orx rats

To determine whether the increase in cortical AP concentration induced by foot shock, isoniazid or FG 7142 was attributable to increased synthesis of this steroid in the brain or to an increased supply from peripheral steroidogenic tissues, we performed experiments in rats that had been adrenalectomized

(Adx)/orchiectomized (Orx) 3 weeks before. As expected, the plasma concentration of AP (Figure 4b) and corticosterone (data not shown) were markedly reduced (−84% and −90%, respectively) in Adx/Orx rats relative to those in sham-operated animals. Moreover, the cortical concentration of AP was also reduced (−42%) in Adx/Orx rats (Figure 4a) although less prominently than in plasma. Foot shock, isoniazid or FG 7142 failed to increase cortical or plasma AP, or plasma corticosterone (data not shown), in Adx/Orx rats killed immediately or 40 or 30 min after the respective treatment (Figure 4a,b).

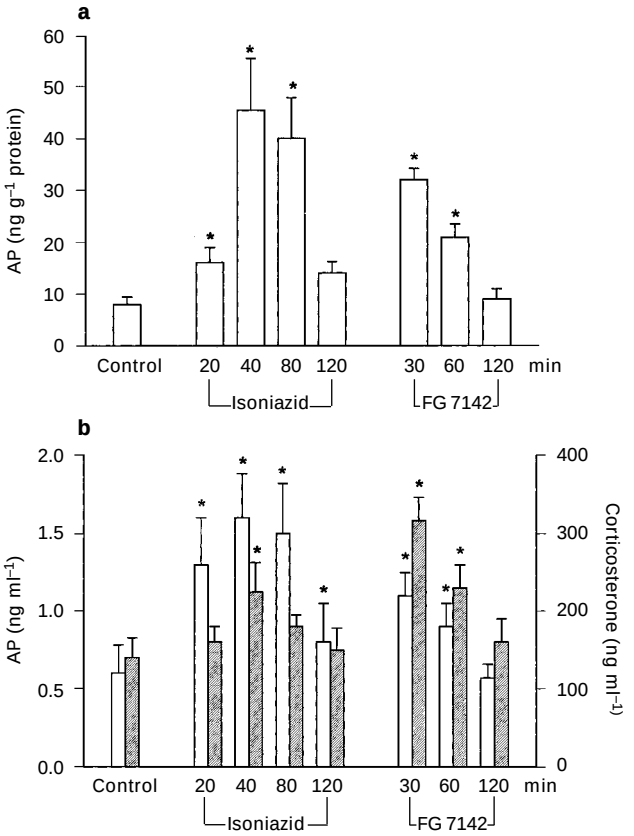

Figure 2 Time course of the changes in (a) cerebrocortical AP concentration and (b) plasma AP (open columns) and corticosterone (hatched columns) levels (b) induced by isoniazid or FG 7142. Handling-habituated rats were treated with isoniazid (375 mg kg^{-1}, s.c.) or FG 7142 (15 mg kg^{-1}, i.p.) and killed at the indicated times thereafter either by focused microwave irradiation to the head (a) or by guillotine (b). Data are means ± s.e.mean of 8 to 10 rats. $*P<0.05$, vs respective control (vehicle-treated handling-habituated rats).

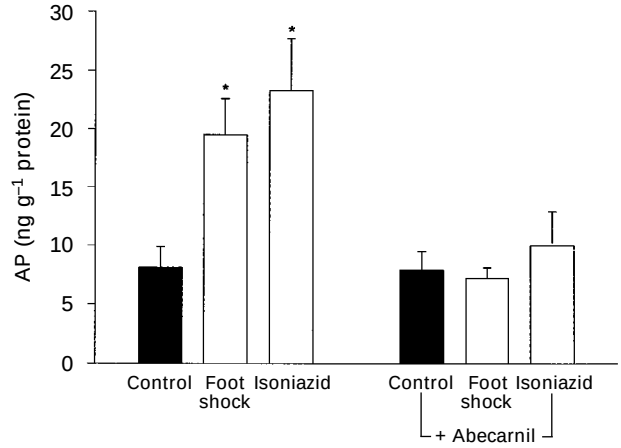

Figure 3 Effects of abecarnil on the increase in cerebral cortical AP concentration induced by foot shock or isoniazid. Handling-habituated rats were treated with abecarnil (0.3 mg kg^{-1}, i.p.) or vehicle 30 min before foot shock or 10 min before injection of isoniazid (375 mg kg^{-1}, s.c.); they were killed immediately after foot shock or 30 min after isoniazid injection. Data are means ± s.e.mean of six rats. $*P<0.05$ vs respective control.

Discussion

We have shown that physiological and pharmacological conditions that decrease brain GABAergic transmission are associated with an increase in the brain content of AP, the most potent endogenous positive allosteric modulator of $GABA_A$ receptors (Majewska *et al*., 1986; Peters *et al*., 1988; Majewska, 1992; Lambert *et al*., 1995; McCauley *et al*., 1995; Concas *et al*., 1996). Thus, transient inhibition of GABAergic transmission in brain elicited either by the GABA synthesis inhibitor isoniazid, by FG 7142, a negative allosteric modulator of $GABA_A$ receptors, or by foot shock triggers a marked and time-dependent increase in the concentration of AP in the cerebral cortex that peaks between 30 and 40 min and returns to baseline by 120 min. This transient increase in brain AP induced by isoniazid, FG 7142 or foot shock is associated with an increase in the plasma concentration of this neuroactive steroid. This latter observation, together with the fact that concentrations of the AP precursors pregnenolone and progesterone are increased by the same treatments in both brain and plasma (Barbaccia *et al*., 1996a; Biggio *et al*., 1996), sug-

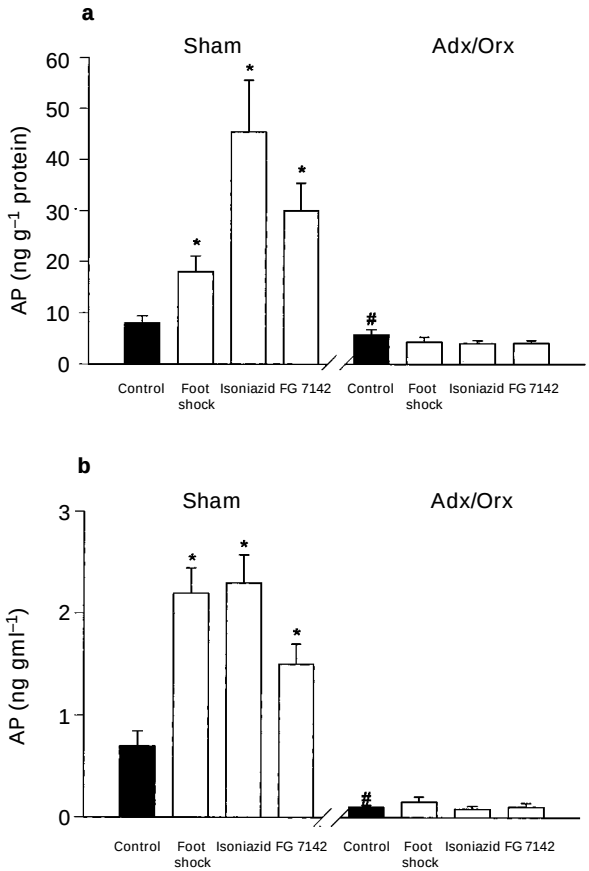

Figure 4 Effects of foot shock, isoniazid or FG 7142 on the concentration of AP in (a) the cerebral cortex and (b) plasma of adrenalectomized/orchiectomized (Adx/Orx) rats. Rats were subjected to Adx/Orx 3 weeks before exposure to foot-shock, isoniazid (375 mg kg^{-1}, s.c.) or FG 7142 (15 mg kg^{-1}, i.p.). The animals were killed immediately or 40 and 30 min after the respective treatment. Data are means ± s.e.mean of eight rats. $*P<0.05$ vs respective control. $\#P<0.05$ vs control sham-operated rats.

gests that the increase in brain AP is attributable to an increased peripheral production or release of AP. This conclusion is supported by the observation that isoniazid, FG 7142 and foot shock each failed to increase AP in both brain and plasma of Adx/Orx rats. The increase in plasma steroid concentrations concomitant with a decrease in GABAergic transmission as well as the antagonism of the foot shock and isoniazid-induced increase in AP in brain and plasma by abecarnil, a positive allosteric modulator of $GABA_A$ receptors, are consistent with the knowledge that GABA inhibits the release of corticotropin-releasing hormone from the hypothalamus (Calogero *et al*., 1988; Owens *et al*., 1991).

Foot shock, isoniazid and FG 7142 each increased brain cortical AP to a similar extent. However, foot shock differed from isoniazid and FG 7142 in the extent to which the plasma corticosterone was increased and in the kinetics of this increase with respect to that of AP, both in plasma and brain. While the peak of plasma corticosterone increase reflects the reduction of $GABA_A$ receptor function elicited by all these treatments (Biggio *et al*., 1984; 1990; Corda & Biggio, 1986; Concas *et al*., 1988; 1996; Serra *et al*., 1990), the peak of brain and plasma AP increase elicited by foot shock appears to correlate with the recovery of $GABA_A$ receptor function that follows the initial reduction, as already observed after acute stress caused by CO_2 inhalation (Barbaccia *et al*., 1996b).

The failure of foot shock stress to increase brain AP content in Adx/Orx rats differs from previous results (Purdy *et al*., 1991) showing that swim stress still increases the amount of AP in the brain of Adx rats. Possible reasons for this apparent discrepancy include the difference in the type of stressor (swim stress vs mild foot shock), and in the method of killing (guillottine vs focused microwave irradiation, the latter of which minimizes *post mortem* steroid metabolism.) Moreover, one should also consider the difference in the surgical procedure i.e. Adx in the previous study (Purdy *et al*., 1991) vs Adx/Orx in the present study, that was used because GABAergic transmission appears to modulate the hypothalamic-pituitary-gonadal (HPG) axis (Masotto *et al*., 1989) in addition to the hypothalamic-pituitary adrenal axis. However, in both conditions, i.e. in Adx or Adx/Orx rats, the extent of AP reduction was greater in plasma than in brain, supporting the notion that the basal amount of AP in the brain (about 50% in our study) does not originate in peripheral steroidogenic tissues and, although could be produced directly in brain, does not seem to be increased by a reduced $GABA_A$ receptor function elicited by foot-shock, isoniazid and FG 7142. Thus, our observations with handling-habituated rats suggest that acute stress and inhibitors of GABAergic transmission similarly increase brain AP concentrations probably by removing the negative GABAergic control on the HPA and/or HPG axis. Accordingly, potentiation of $GABA_A$ receptor function by abecarnil prevents the stress or isoniazid-induced increase in AP in the brain probably by attenuating HPA responsiveness to stress (Stephens *et al*., 1990).

Our finding that the cortical concentration of AP and its precursors are higher in naive than in handling-habituated rats, whereas the plasma concentration of this neurosteroid, as well as those of pregnenolone, progesterone and corticosterone, are similar in the two experimental groups, suggests that the excess of cortical AP in naive animals is not derived from activation of the HPA axis, but rather from an increased production or decreased metabolism of AP in brain. Brain cells express the enzymes 5α-reductase and 3α-hydroxysteroid dehydrogenase (Celotti *et al*., 1992; Cheney *et al*., 1995), which catalyze the production of AP from progesterone and the oxidation of AP to 5α-dihydroprogesterone. Because naive rats show a reduced GABAergic transmission relative to handling-habituated animals (Biggio *et al*., 1984, 1990; Concas *et al*., 1988), these results are apparently at odds with the failure of foot-shock, isoniazid and FG 7142 to increase AP in Adx/Orx rats. One way to address this point would be to evaluate whether the cortical concentrations of AP differ between handling-habituated and naive Adx/Orx rats. However, in our experience, Adx/Orx rats are less likely to become handling-habituated than sham-operated or intact rats, consistent with a role for the adrenal gland in habituation to handling and, therefore, in the adaptation to stress. On the basis of the present evidence one may conclude that the reduction of $GABA_A$ receptor function in naive rats is not directly linked to the modulation of brain AP production/metabolism, and the increase of plasma steroids is not detected in these animals because it takes at least a few minutes for ACTH to be released and stimulate the adrenals. Alternatively the stress induced by handling in naive rats elicits a reduction of GABAergic transmission at specific synapses other than those involved in the modulation of hypothalmic corticotropin releasing hormone, which may play a permissive role in increasing the availability of AP in brain.

In conclusion, we have shown that the brain content of AP, one of the most potent endogenous positive modulators of $GABA_A$ receptors, increases markedly in response to pharmacological treatments that selectively reduce GABAergic transmission or to acute foot-shock. The increase in brain AP induced by isoniazid, FG 7142 or foot shock appears to result mainly from the removal of an inhibitory GABAergic control of the HPA and/or HPG axis, as shown by the failure of these treatments to increase the concentration of AP in brain or plasma in Adx/Orx rats. On the other hand the observation that the stress caused by handling in naive rats is associated with an increase in AP in the brain but not in plasma indicates that factors other than the removal of inhibitory GABAergic control over the HPA axis may play a role in neurosteroid homeostatis in the brain and supports previous studies (Le Goascogne *et al*., 1987; Barbaccia *et al*., 1992; Corpèchot *et al*., 1993; Mathur *et al*., 1993; Cheney *et al*., 1995) showing that steroidogenesis occurs in brain, where it appears to be independent of the pituitary factors that control adrenal/gonadal steroidogenesis. Finally, the marked, but transient, increase in AP, as well as in allotetrahydrocorticosterone (Barbaccia *et al*., 1996a,b; Biggio *et al*., 1996), in brain elicited by these treatments may subserve physiological functions. Thus, it is appealing to speculate that AP, which increases $GABA_A$ receptor function with greater efficacy under conditions in which GABA concentrations at GABAergic synapses are reduced (Concas *et al*., 1996), plays a role in the adaptation to stress by limiting the extent and duration of the inhibition of GABAergic transmission induced by acute stress.

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