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Differential Blockade of γ-Aminobutyric Acid Type A Receptors by the Neuroactive Steroid Dehydroepiandrosterone Sulfate in Posterior and Intermediate Pituitary

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ABSTRACT

Dehydroepiandrosterone sulfate (DHEAS) is a neuroactive steroid with antagonist action at γ-aminobutyric acid type A ($GABA_A$) receptors. Patch-clamp techniques were used to investigate DHEAS actions at $GABA_A$ receptors of the rat pituitary gland at two distinct loci: posterior pituitary nerve terminals and intermediate pituitary endocrine cells. The GABA responses in these two regions were quite different, with posterior pituitary responses having smaller amplitudes and desensitizing more rapidly and more completely. DHEAS blockade of $GABA_A$ receptors in the two regions also was different. In posterior pituitary, a site with an apparent dissociation constant of 15 μM accounted for most of the blockade, but a small fraction of blockade may be related to a site with a dissociation constant in the nanomolar range. In the intermediate lobe, DHEAS sensitivities in the nanomolar and micromolar ranges were clearly evident, in proportions that varied widely from cell to cell. Regardless of whether the GABA response of a cell was highly sensitive or weakly sensitive to DHEAS, GABA alone evoked currents that were indistinguishable in terms of amplitude, desensitization kinetics, and GABA sensitivity. Thus, the structural elements responsible for DHEAS blockade have a highly selective impact on receptor function. $GABA_A$ receptors with nanomolar sensitivity to DHEAS have not been described previously. This suggests that DHEAS may have an important role in the modulation of neuropeptide secretion, and the diverse properties of $GABA_A$ receptors in the rat pituitary provide mechanisms for selective regulation of the different peptidergic systems of this gland.

In endocrine cells of the pituitary intermediate lobe (IL), γ-aminobutyric acid type A ($GABA_A$) receptor-specific agonists modulate the release of α-melanocyte-stimulating hormone (Tomiko et al., 1983; Taraskevich and Douglas, 1985). In the nerve terminals of the posterior pituitary (PP), $GABA_A$ receptor activation alters the release of oxytocin and vasopressin (Dyball and Shaw, 1978; Fjalland et al., 1987; Saridaki et al., 1989). In both IL (Demeneix et al., 1986; Taleb et al., 1987; Schneggenburger and Konnerth, 1992) and PP (Zhang and Jackson, 1993), the receptors have many of the properties of classic neuronal $GABA_A$ receptors. The channels are selectively permeable to Cl^-, muscimol acts as an agonist, and the responses are blocked by bicuculline and picrotoxin. IL $GABA_A$ receptors possess a pure type 2 benzodiazepine-binding site, and ribonuclease protection assays have shown that the IL contains mRNA encoding for the α2, α3, β1, β3, γ2s, and γ1 $GABA_A$ receptor subunits (Berman et al., 1994). Sensitivity to benzodiazepines and insensitivity to zinc indicate that PP $GABA_A$ receptors contain γ subunits (Zhang and Jackson, 1994b). These different molecular and pharmacological properties determine how GABA and $GABA_A$ receptor-specific drugs regulate different peptide systems in the pituitary gland.

Neuroactive steroids modulate the responsiveness of $GABA_A$ receptors in many preparations, and this represents an important nongenomic action of steroids (Harrison and Simmonds, 1984; Paul and Purdy, 1992; Gee et al., 1995). Allopregnanolone (5α-pregnan-3α-hydroxy-20-one) and alphaxalone have been shown to act as positive $GABA_A$ receptor modulators in the PP (Zhang and Jackson, 1994a). In the IL, allopregnanolone also enhances $GABA_A$ receptor-mediated responses, and the neuroactive steroid pregnenolone sulfate (5-pregnen-3α-ol-20-one sulfate) antagonizes them (Poisbeau et al., 1997). These findings raise the possibility that neuroactive steroid modulation of neuropeptide release plays a role in some of the endocrinological transitions mediated by these peptidergic systems. Furthermore, neuroactive steroid potencies vary between brain regions and species (Gee et al., 1995; Nguyen et al., 1995). This adds another dimension to

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ABBREVIATIONS: DHEAS, dehydroepiandrosterone sulfate; GABA, γ-aminobutyric acid; IL, intermediate lobe; PP, posterior pituitary; aCSF, artificial cerebrospinal fluid.

neuroactive steroid signaling by allowing for differential control over peptidergic versus synaptic processes and, possibly, differential control between various peptidergic systems.

Dehydroepiandrosterone sulfate (DHEAS) is a neuroactive steroid that has been the subject of wide-ranging discussions regarding potential roles in cognitive function, aging, stress, and development (Baulieu and Robel, 1996; Bastianetto and Quirion, 1997). In contrast to many neuroactive steroids that enhance the responses of $GABA_A$ receptors, DHEAS blocks $GABA_A$ receptors in a number of preparations (Majewska et al., 1990; Spivak, 1994; Souza and Ticku, 1997). In the present study, we investigated the actions of DHEAS at $GABA_A$ receptors in slices prepared from the rat pituitary gland (Jackson et al., 1991; Schneggenburger and Konnerth, 1992), focusing on both the peptidergic nerve terminals of the PP and the endocrine cells of the IL. DHEAS inhibits GABA responses in both PP and IL but with a concentration dependence indicative of multiple binding sites. Variations in the DHEAS sensitivity of endocrine cells within the IL suggest the presence of multiple molecular forms of $GABA_A$ receptors. This would allow DHEAS to influence neuropeptide release over a broad range of concentrations and possibly modulate different peptide systems selectively.

Materials and Methods

Pituitary Slices. Slices of rat pituitary gland were prepared as described previously (Jackson et al., 1991). Male Sprague-Dawley rats 4 to 6 weeks old were sacrificed by decapitation after CO_2-induced narcosis. The pituitary gland was quickly removed and placed into ice-cold artificial cerebrospinal fluid (aCSF) consisting of 125 mM NaCl, 4 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM glucose, pH 7.3, bubbled with a mixture of 95% O_2/5% CO_2 (carbogen). The pituitary gland was glued to a cutting block, with the posterior lobe facing upward, and immersed in chilled aCSF. Slices 70 to 80 μm thick were then cut with a Vibratome. Slices were either kept in carbogen-bubbled aCSF or transferred to a recording chamber. Recordings were made with continuous perfusion of carbogen-bubbled aCSF, while being viewed with a DIC microscope at 600×. Nerve terminals in the PP ranging in size from 5 to 15 μm in diameter were selected for patch-clamp recording. IL endocrine cells at the perimeter of the slice were readily identified by appearance and location (Schneggenburger and Konnerth, 1992).

Drug Application. Drugs were dissolved in a solution consisting of 140 mM NaCl, 3.5 mM KCl, 10 mM glucose, 1.25 mM Na_2HPO_4, 2 mM $MgCl_2$, 2 mM $CaCl_2$, and 20 mM HEPES, pH 7.3, and applied focally by one of two methods. In one method, a drug-containing patch pipette was positioned ~5 μm from the cell or terminal under recording, and the drug was ejected with pressure (12 p.s.i.) from a Picospritzer (General Valve Corp., Fairfield, NJ). In the second method, drugs were applied by gravity-feed through a ~100-μm-diameter tube positioned close to the cell or terminal under recording, with different solutions selected from one of seven channels by electrically operated valves. Between drug applications, the system fed a drug-free solution to maintain constant flow and reduce hydrodynamic switching artifacts. This also hastened recovery after drug application. Because the Picospritzer delivers drugs more rapidly than the multibarrel gravity-feed system, we compared responses to GABA applied by the two methods. Each method resulted in responses with the same amplitude in either PP nerve terminals or IL endocrine cells (Fig. 1). The multibarrel application system was chosen for most experiments because this method makes it easy to test several drugs concentrations in the same recording.

All drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO), except DHEAS, which was purchased from Research Biochemicals International (Natick, MA). In general, responses to 50 μM GABA were tested at regular intervals to check the stability of control responses. Previous work from this laboratory has shown that GABA responses in PP showed no run-down (Zhang and Jackson, 1994b), and responses in IL examined here showed a similar stability. Application times of 1 to 2 s were used for PP terminals, and a time of 8 s was used for for IL cells. These application times were based on differences in desensitization kinetics in the two preparations (see *Results*). Control GABA responses recovered completely by 1 min after DHEAS application ended, so 1 min was allowed between tests with different drugs and concentrations. In experiments in which longer times were allowed, no difference was observed. In studies of concentration dependence, different concentrations were selected in random sequences.

Except where noted, various concentrations of antagonists were applied simultaneously with 50 μM GABA as premixed solutions. When 100 μM DHEAS alone was applied 2 to 3 min before the application of a mixture of 50 μM GABA and 100 μM DHEAS, the degree of blockade (~75%) was indistinguishable from the degree of blockade without pretreatment in both PP ($n = 4$) and IL ($n = 4$). Thus, the action of DHEAS was sufficiently rapid to obtain full effects with simultaneous presentations of DHEAS and GABA.

Electrophysiological Recording. Patch-clamp recordings from nerve terminals and endocrine cells in pituitary slices were made at room temperature according to standard methods (Hamill et al., 1981). All recordings were made with a holding potential of −70 mV. Patch electrodes with resistances between 2 and 5 MΩ were made from borosilicate glass (1.1 mm I.D., 1.7 mm O.D.; Garner Glass, Claremont, CA) and filled with a solution consisting of 130 mM KCl, 10 mM HEPES, 10 mM EGTA, 2 mM $MgCl_2$, and 2 mM MgATP, pH 7.2. Tight-seal intracellular recordings were achieved with series resistances ranging from 3 to 15 MΩ. Whole-terminal and whole-cell current was recorded under voltage clamp with an Axopatch 200 patch-clamp amplifier (Axon Instruments, Foster City, CA).

Data Acquisition and Analysis. Data acquisition and analysis were carried out with pCLAMP6 software (Axon Instruments) on a personal computer. Curve fitting was performed with the computer program ORIGIN (Microcal Software, Northampton, MA), and statistical analysis was carried out with Sigma Stat 2.0 (Jandel Statistical Software, Erkrath, Germany).

Concentration-inhibition plots (for the blockade of GABA responses by DHEAS) were fitted to models with one or two binding sites. The one-binding-site model had the form

$$I = \frac{100}{1 + C/K} \tag{1}$$

where I is the response as percent of control, K is the apparent dissociation constant, and C is blocker concentration. These fits were compared with fits to a two-binding-site model of the form

$$I = \frac{X_1}{1 + C/K_1} + \frac{100 - X_1}{1 + C/K_2} \tag{2}$$

where K_1 and K_2 are the apparent dissociation constants of two different sites, and X_1 is the percentage of receptor with an apparent dissociation constant of K_1. The statistical significance of fits to these equations was evaluated by the χ^2 goodness-of-fit test (Bevington and Robinson, 1992), using a cutoff probability of .05.

Concentration-response plots (for activation of receptors by GABA) were fitted to the Hill equation

$$I = \frac{100(C/K)^n}{1 + (C/K)^n} \tag{3}$$

where K is the apparent dissociation constant and n is the Hill coefficient.

Open probability at the peak of a response was measured by

fluctuation analysis. According to the binomial distribution, the mean current is Nip, where N is the number of channels, i is the single channel current, and p is the open probability. The variance is $Ni^2p(1 - p)$, and the variance to mean ratio is $i(1 - p)$. Because i is known in both PP (Zhang and Jackson, 1993) and IL (Taleb et al., 1987), p can be calculated. We determined the variance from a flat segment of data at the peak of the response typically lasting 20 to 100 ms. Baseline variance was determined from current recorded before drug application in the same experiment and subtracted from the variance at the peak.

Unless otherwise stated, arithmetic means from different groupings were compared using the Student's t test, with a cutoff probability of .05.

Results

GABA Responses in PP and IL. GABA responses in PP terminals are mediated by $GABA_A$ receptors (Saridaki et al., 1989; Zhang and Jackson, 1993). The pharmacology of IL $GABA_A$ receptors has been described previously (Demeneix et al., 1986; Schneggenburger and Konnerth, 1992), and we verified this by showing that the current evoked by 50 μM GABA was blocked completely by 100 μM bicuculline methbromide ($n = 8$) and 89 ± 8% blocked ($n = 8$) by 100 μM picrotoxinin.

With both of the application methods used in this study, responses to GABA (50 μM) in the PP were strikingly different from those in IL (Fig. 1). The response amplitudes were ~10- to 15-fold greater in IL endocrine cells than in PP nerve terminals; responses in both structures decayed in the presence of sustained GABA application, but in the IL, this decay was ~18-fold slower. The basic parameters of these responses are summarized in Table 1. (The high-sensitivity and low-sensitivity groupings of IL cells in this table are based on

TABLE 1

Response characteristics and kinetic parameters

Responses to 50 μM GABA were compared between PP and IL. Responses in IL cells were divided into two groups based on amount of blockade by 0.01 μM DHEAS. Cells in which DHEAS reduced GABA responses to <30% of control were classified as high sensitivity; cells in which GABA responses remained within 60% of control were classified as low sensitivity. D/P was ratio of the end point amplitude of current after desensitization was complete (D) to peak response (P).

	Amplitude	D/P	Desensitization Half-time	n
	pA		*ms*	
PP[a]	45 ± 10	0.11 ± 0.05	300 ± 70	7
IL[b]				
High sensitivity	530 ± 80	0.25 ± 0.06	5420 ± 880	6
Low sensitivity	740 ± 160	0.29 ± 0.06	5160 ± 390	7

[a] Between pooled IL data and PP, all quantities showed statistically significant differences.

[b] Between high and low DHEAS sensitivity IL cells, no quantities showed statistically significant differences.

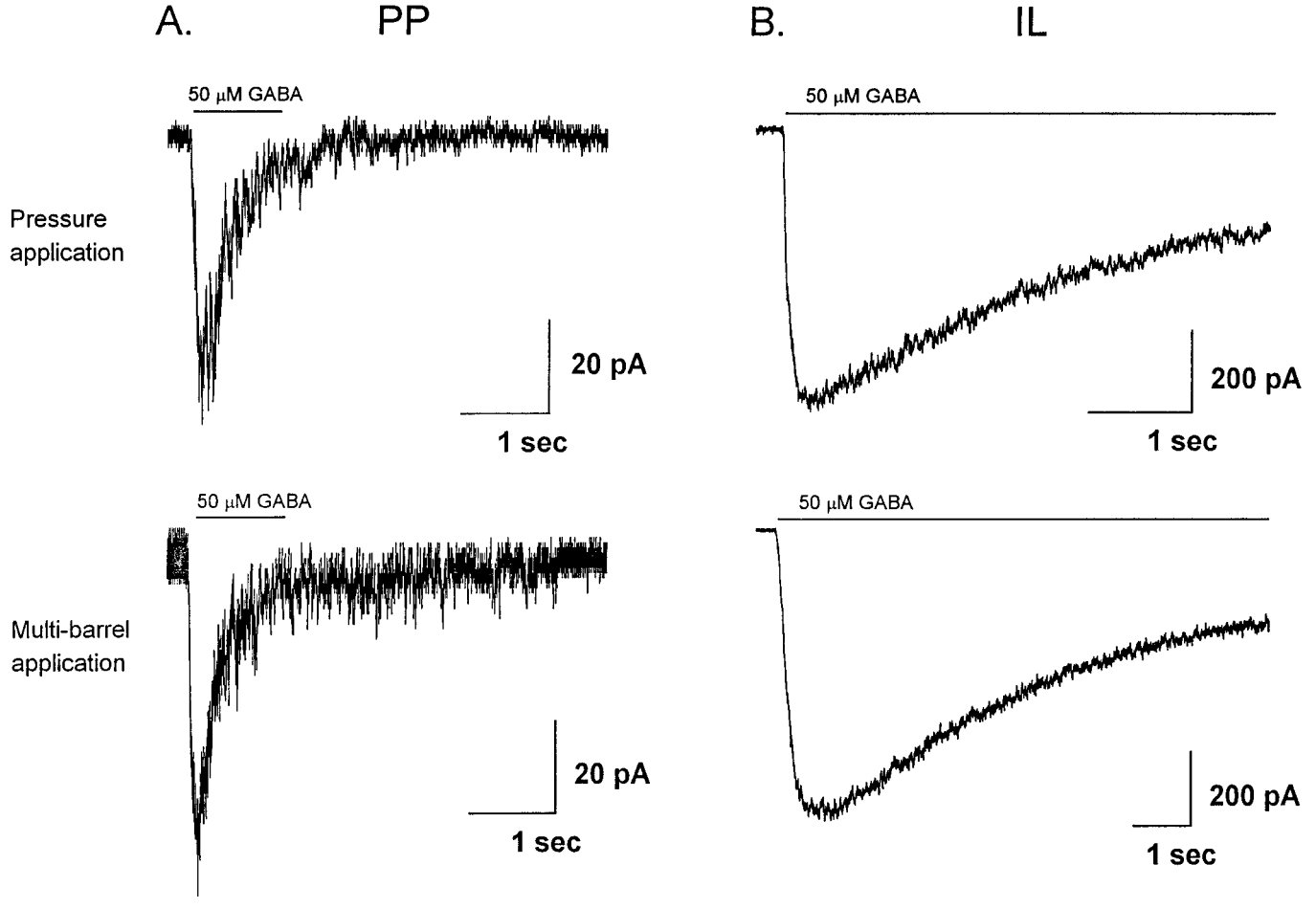

Fig. 1. Responses to GABA in voltage-clamped PP nerve terminals and IL endocrine cells, with pressure and gravity-feed application. GABA (50 μM) was applied for the times indicated (bar). In all experiments, the duration of GABA application was sufficient to desensitize the receptors. The two drug-application methods gave similar results when applied to the same cell, as judged by the Mann-Whitney rank sum test (in PP: Picospritzer, $n = 4$; multibarrel, $n = 10$; in IL: Picospritzer, $n = 3$; multibarrel, $n = 8$). Response properties are summarized in Table 1.

variations in DHEAS sensitivity explained below.) In addition, three cells in the anterior pituitary were tested with 50 μM GABA. Responses were very small (3–6 pA), so the anterior pituitary was not studied further.

As noted above in *Materials and Methods*, drug application by pressure and gravity-feed gave similar results within each pituitary region (Fig. 1). Furthermore, response rise times were clearly more rapid than the decays seen with continuous drug application. This makes it unlikely that drug presentation distorts response amplitudes or desensitization kinetics and thus indicates that the differences between PP and IL responses can be attributed to receptor properties. The low-amplitude responses in PP are consistent with earlier reports from this laboratory (Zhang and Jackson, 1993, 1995), and the larger amplitudes of responses in IL are comparable to those seen in dissociated IL cells (Poisbeau et al., 1997). PP and IL $GABA_A$ receptor channels have similar single-channel conductances (Taleb et al., 1987; Zhang and Jackson, 1993). Capacitance measurements taken during these experiments gave mean values of 7.2 pF in PP and 8.3 pF in IL, indicating that the membrane areas are comparable. Fluctuation analysis (see *Materials and Methods*) gave open probabilities at the peak of the response as .82 ± .05 (n = 6) in PP and .76 ± .07 (n = 10) in IL. Thus, the different response amplitudes most likely reflect differences in receptor density.

Table 1 summarizes the comparison of the kinetic parameters associated with GABA responses in the two regions. As noted above, GABA responses in IL were larger and desensitized more slowly than those in PP. In all experiments, GABA application was maintained for a sufficient duration to desensitize receptors, and the half-time for responses to decay was taken as a simple quantitative index of this process. Curve fits of exponential functions to these decay processes were used to estimate the final end point level of desensitization, and the ratio of this value to the peak amplitude (D/P in Table 1) provides a quantitative indication of the extent of desensitization relative to peak. This ratio was significantly lower in PP, indicating that desensitization of PP $GABA_A$ receptors was more complete than desensitization of IL $GABA_A$ receptors.

DHEAS Blockade of GABA Responses in PP. DHEAS was an effective antagonist at $GABA_A$ receptor-mediated responses in PP nerve terminals (Fig. 2). The response to 50 μM GABA was nearly completely blocked by 100 μM DHEAS, and as noted, the GABA response recovered fully within 1 min after DHEAS removal. DHEAS alone at this concentration had no effect. Figure 2 shows that a lower concentration of DHEAS (0.1 μM) blocked the GABA response by ~50%.

The concentration dependence of DHEAS action at $GABA_A$ receptors is shown more fully in Fig. 3. This concentration-inhibition plot shows increasing blockade as DHEAS concentration increased from 30 pM to 100 μM. The best-fitting two-binding-site model was drawn through the data. The one-binding-site model (fit not shown) gave a χ^2 value that was ~7-fold higher than that obtained from the two-binding-site model. Furthermore, the two-binding-site model satisfied the χ^2 test for goodness-of-fit to these data with $P > .8$. These data clearly show a low-affinity site and provide some indication for the existence of a second high-affinity site. If there are two sites, the low-affinity site with an apparent dissociation constant of 15 ± 4 μM predominates in determining the concentration dependence of blockade (Table 2). A second site, implied by the weak blockade at 1000-fold lower concentrations, has an apparent dissociation constant of 0.21 ± 0.15 nM and accounts for only 28 ± 3% of the response to GABA.

DHEAS Blockade of GABA Responses in IL. DHEAS also was an effective antagonist at $GABA_A$ receptors in IL endocrine cells. Blockade was reversible, and responses to GABA recovered within 1 min. In contrast to the PP, in the IL, the potency of blockade varied enormously for a given DHEAS concentration. Figure 4 shows the effect of 0.01 μM DHEAS on GABA responses in two different IL cells. In one cell, the response was reduced to 15% of control (Fig. 4A), and in another cell, the response was reduced to 95% of control (Fig. 4B). To illustrate this variability more completely, data from 24 cells were plotted in scatter form without averaging, with DHEAS concentrations ranging from 30 pM to 100 μM (Fig. 5). This plot shows that between 1 nM and 10 μM DHEAS, the variation in potency was especially large. Repeated tests of a given DHEAS concentration in a single IL cell gave reproducible amounts of blockade. Furthermore, there was much less variability in blockade of the GABA responses in PP nerve terminals. Thus, the scatter in Fig. 5 is greater than can be accounted for by experimental error.

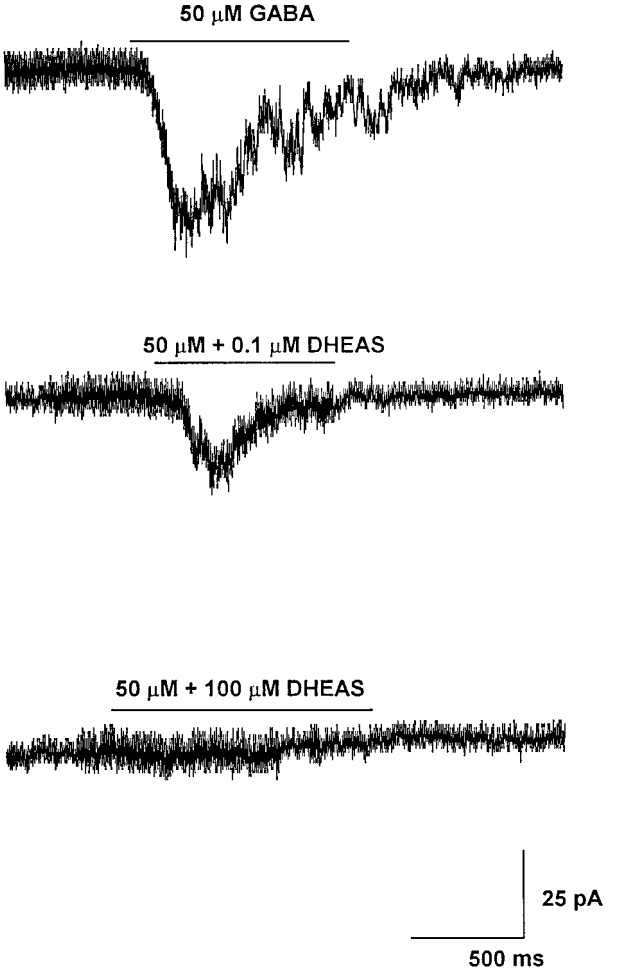

Fig. 2. Blockade of PP GABA responses by DHEAS. GABA (50 μM) was applied alone, with 0.1 μM DHEAS and with 100 μM DHEAS.

One possible explanation for the large variability in DHEAS sensitivity is that cells are heterogeneous and contain variable proportions of two $GABA_A$ receptors with high DHEAS sensitivity and low DHEAS sensitivity. To explore this possibility, we focused on the portion of the concentration-inhibition curve with the greatest variability. Figure 4 indicates that the blockade of IL $GABA_A$ receptors by 0.01 μM DHEAS varies widely. Furthermore, Fig. 5 shows that 0.01 μM is in a highly variable part of the concentration range. Therefore, the degree of blockade of GABA responses by this concentration was used as the basis for dividing IL cells into the following three groups: 1) cells with GABA responses blocked to less than 60% of control were classified as having low DHEAS sensitivity. Cells with GABA responses blocked to between 30% and 60% of control were classified as having intermediate DHEAS sensitivity. Cells with GABA responses blocked to less than 30% of control were classified as having high DHEAS sensitivity. According to these criteria, 57% of the cells were of low, 10% were of intermediate, and 33% were of high DHEAS sensitivity.

The data from high- and low-sensitivity IL cells were used to construct two separate concentration-inhibition plots (Fig. 6). In both cases, the one-binding-site model fitted the data poorly. The χ^2 value was reduced 6- or 7-fold by fitting to the two-binding-site model (solid curves in each graph). The χ^2 goodness-of-fit test for the two-binding-site model gave values of $P > .8$ and $P > .20$ for the high- and low-sensitivity data, respectively. For the high-affinity site, these fits yielded apparent dissociation constants of 0.23 ± 0.05 and 1.8 ± 1.5 nM in the high- and low-sensitivity cells, respectively. The low-affinity binding sites had apparent dissociation constants of 0.7 ± 0.6 and 5 ± 3 μM for the high- and low-sensitivity cells, respectively. In the high-sensitivity cells, the high-affinity binding site accounted for 89 ± 3% of the inhibition, and in the low-sensitivity cells, the high-affin-

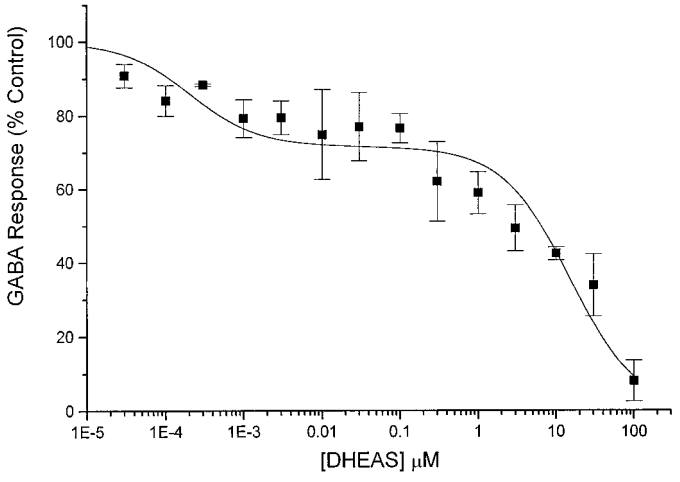

Fig. 3. DHEAS concentration-inhibition plot in PP. Control responses to 50 μM GABA and responses to GABA plus DHEAS were recorded in the same nerve terminal. All responses were then normalized to the control GABA response and plotted. Data from 17 nerve terminals were used, with each DHEAS concentration tested in 3 to 6 nerve terminals. Mean ± S.E. values were plotted. The curve is the best fitting two-binding-site model (see *Materials and Methods*). The parameters obtained from this fit are presented in Table 2. The two-binding-site model satisfied the χ^2 goodness-of-fit test, and the one-binding-site model did not.

TABLE 2

Apparent dissociation constants for DHEAS

Parameters were obtained from fits of the two-binding-site model (see *Materials and Methods*) to DHEAS concentration-inhibition plots. PP fit is shown in Fig. 3. IL fits are shown in Fig. 6. K_1 and K_2 are apparent dissociation constants for two sites, and X_1 is percentage of block contributed by site 1. In each fit, the two-binding-site model satisfied the χ^2 goodness-of-fit test, and the one-binding-site model did not. High- and low-sensitivity IL cells were classified on basis of degree of blockade by 0.01 μM DHEAS of GABA responses to <30% or above 60% of control (see text).

	K_1	K_2	X_1
	nM	*μM*	*% site 1*
PP	0.21 ± 0.15	15 ± 4	28 ± 3
IL			
High sensitivity	0.23 ± 0.04	0.7 ± 0.6	89 ± 3
Low sensitivity	1.8 ± 1.5	5 ± 3	39 ± 9

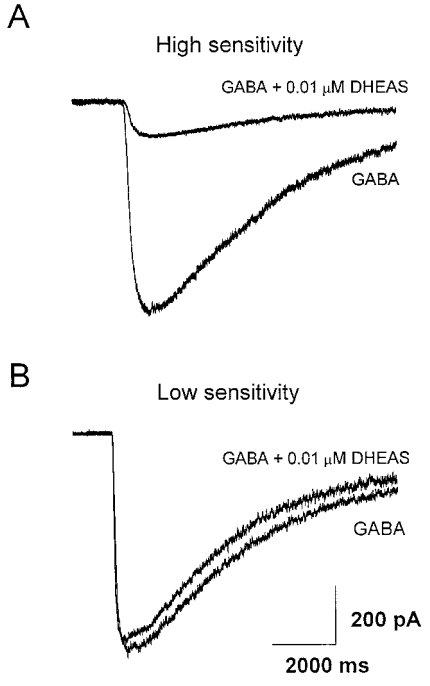

Fig. 4. Variable blockade of IL GABA responses by DHEAS. Responses to 50 μM GABA are shown together with responses to GABA plus 0.01 μM DHEAS. A, in a cell with high DHEAS sensitivity, the response was blocked by ~90%. B, in a cell with low DHEAS sensitivity, the response was blocked by less than 10%.

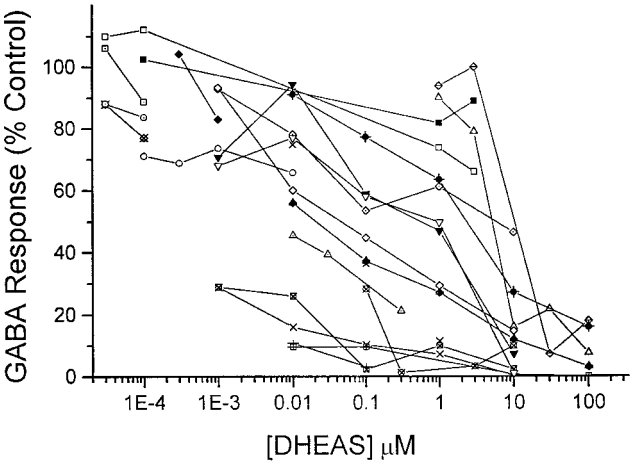

Fig. 5. Concentration-inhibition scatterplot for DHEAS blockade of GABA responses in IL. Responses were normalized to the control GABA response from the same cell. Points from each measurement were plotted individually to illustrate the variability. Blockade was most variable between 0.001 and 10 μM.

ity binding site accounted for 39 ± 9% of the inhibition. It is significant that in each plot, two apparent dissociation constants were obtained: one in the nanomolar range and the other in the micromolar range. The relative proportions of nanomolar and micromolar components in these two plots are consistent with the hypothesis that IL cells contain two distinct $GABA_A$ receptor variants, with DHEAS sensitivities differing by ~3 orders of magnitude. The apparent dissociation constants and fractions of high-affinity site are listed in Table 2, together with the results for PP.

Comparisons of High- and Low-Sensitivity Receptors. Because the results above suggested that there are different types of $GABA_A$ receptors in different IL cells, we compared various features of GABA responses after classifying cells on the basis of high sensitivity and low sensitivity to DHEAS. There were no differences evident between the two groups of IL cells on qualitative examination, and quantitative comparisons of response amplitudes and desensitization behavior also show no differences (Table 1). The kinetics of $GABA_A$ receptor-mediated responses in the PP have been analyzed previously and shown to desensitize with simple exponential kinetics (Zhang and Jackson, 1994b). In IL cells with either high or low DHEAS sensitivity, the time courses of desensitization of responses to GABA alone were also well described by a single exponential in most cases (data not shown). The results in Table 1 show that IL cells with high and low sensitivity to DHEAS are indistinguishable in terms of speed and extent of desensitization. (The half-time in the table is taken as a measure of speed, and the ratio D/P of final to peak current is taken as a measure of extent.)

Responses to different concentrations of GABA were measured and used to construct concentration-response plots for IL cells with either high or low DHEAS sensitivity (Fig. 7). Fits to the Hill equation (see *Materials and Methods*) indicated that the apparent dissociation constants for GABA were 55 ± 11 and 66 ± 11 μM and the Hill coefficients were 1.3 ± 0.3 and 1.5 ± 0.3 in the high-sensitivity and low-sensitivity groups, respectively. These values are indistinguishable between the two groups, indicating these two types of receptors differ very specifically with regard to DHEAS sensitivity. Other domains of the receptor with roles in determining GABA sensitivity and response kinetics appear to be unaltered.

We also examined the kinetics of GABA responses blocked ~50% by DHEAS. The responses were generally more complex than the responses to GABA alone and were difficult to interpret quantitatively. The only noticeable feature of these responses was that in both high-sensitivity and low-sensitivity cells, DHEAS made the GABA responses desensitize more

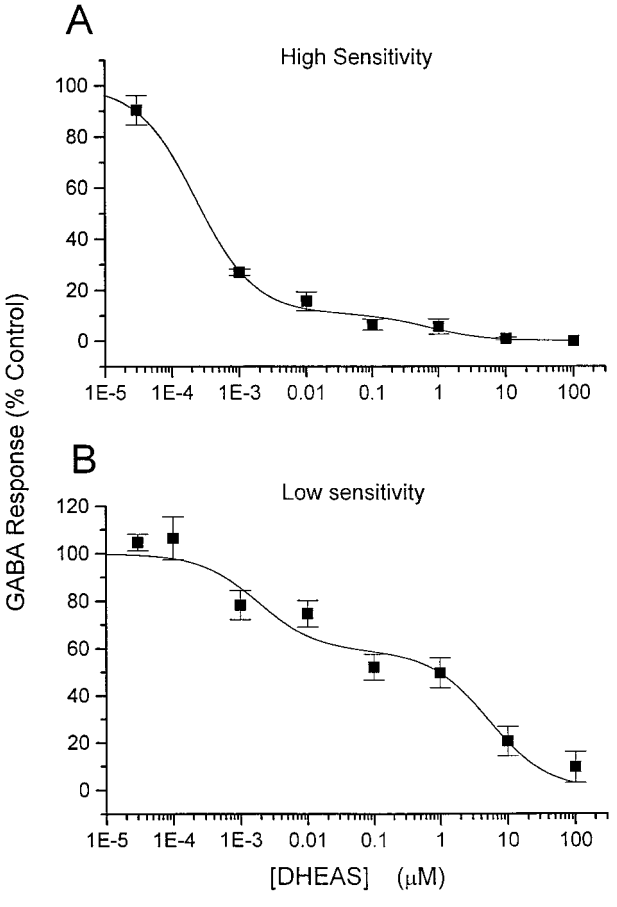

Fig. 6. Concentration-inhibition plots in IL cells with high DHEAS sensitivity (A) and low DHEAS sensitivity (B). Based on blockade by 0.01 μM DHEAS, IL cells were separated into the two groups as described in the text. Fits to the two-binding-site model (see *Materials and Methods*) satisfied the χ^2 goodness-of-fit test in both plots, but fits to the one-binding-site model did not. See Table 2 for parameter values.

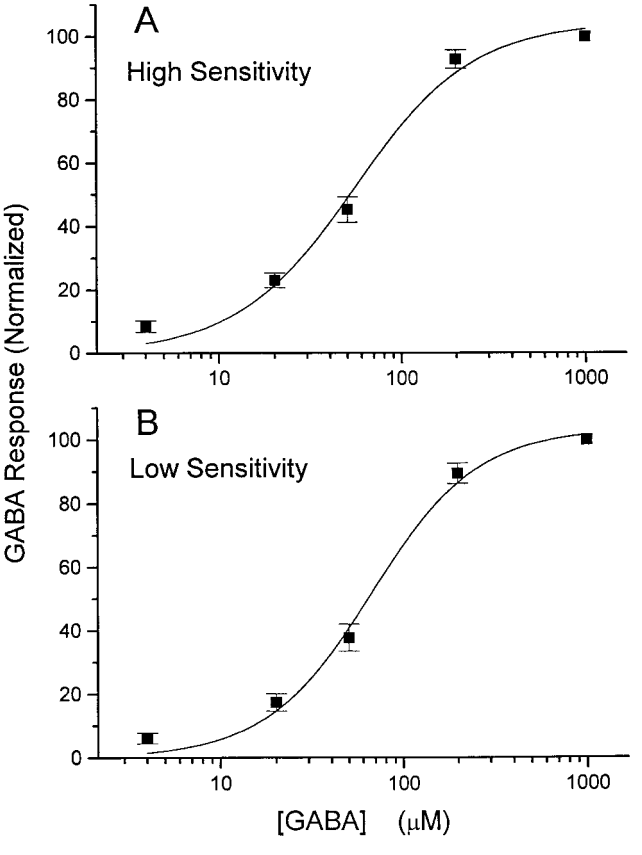

Fig. 7. GABA concentration-response plots from IL cells with high (A) or low (B) DHEAS sensitivity. Responses were normalized to the response to 1 mM GABA. Data for all five concentrations of GABA were averaged from five cells for the high-sensitivity group and eight cells for the low-sensitivity group. Mean ± S.E. values are shown together with the best-fitting Hill equation. The apparent dissociation constants were 55 ± 11 and 66 ± 11 μM for the high-sensitivity and low-sensitivity groups, respectively. The Hill coefficients were 1.3 ± 0.3 and 1.5 ± 0.3, respectively. The extrapolated maximum responses were 1.04 times greater than the 1 mM responses in both groups.

slowly (data not shown). Another neurosteroid antagonist, pregnenolone sulfate, was found to accelerate desensitization in IL cells (Poisbeau et al., 1997). In PP nerve terminals, there was no difference in the kinetic parameters between GABA alone and GABA plus 1 μM DHEAS (data not shown). The decays were well fitted by one exponential in both the presence and absence of DHEAS. In PP, the enhancing neuroactive steroids allopregnanolone and alphaxolone also left unchanged the time constant for desensitization of GABA responses (Zhang and Jackson, 1994a). Thus, in this respect, the antagonist and enhancing neuroactive steroids are similar.

Discussion

These experiments demonstrated that the neuroactive steroid DHEAS has antagonist activity at $GABA_A$ receptors in two distinct parts of the pituitary gland. In IL endocrine cells, multiple DHEAS binding sites were evident with sensitivities in the micromolar and nanomolar ranges. $GABA_A$ receptor properties differed between the PP and IL, as well as within the IL. The sensitivity of these receptors to neuroactive steroids provides a potentially important signaling pathway in the regulation of neuropeptide release.

Differences between PP and IL $GABA_A$ Receptors. $GABA_A$ receptors in PP nerve terminals and IL endocrine cells differed in their basic response characteristics. The current evoked by 50 μM GABA was much larger in IL than in PP. The smaller responses in PP probably reflect a low density of channels. The comparison of response properties revealed that desensitization is faster and more complete in PP than in IL (Table 1). The slow desensitization of IL $GABA_A$ receptors was similar to that observed by Poisbeau et al. (1997) in dissociated rat IL cells. The contrast between slow and fast desensitization of IL and PP $GABA_A$ receptors is reminiscent of the difference between *Drosophila* GABA receptors assembled as homomeric complexes from the Rdl gene product or as heteromeric complexes with β subunits (Zhang et al., 1995). Desensitization is virtually absent in vertebrate receptors where the only α subunit is α6, and removal of the γ subunit results in a large nondesensitizing component of current (Tia et al., 1996). The inclusion of a δ subunit also slows desensitization (Saxena and Macdonald, 1994). Thus, a molecular basis for different desensitization rates in GABA receptors is well established and could account for the differences observed here.

The physiology of GABA-mediated inhibition could be very different in the PP and IL, and the different receptor properties in the two regions may have an adaptive significance. Release of vasopressin and oxytocin from the PP is under the control of rhythmic activity in the hypothalamus (Poulain and Wakerley, 1982). GABA in the PP cannot influence this distant location, so to modulate release, GABA must block action potentials or alter their shape. Under some conditions, GABA may be able to block action potential propagation through the PP nerve terminal arbor (Zhang and Jackson, 1993; Jackson and Zhang, 1995), but the timing would be important because an action potential is a very brief event. The rapid kinetics of the PP $GABA_A$ receptor could produce a brief episode of inhibition, which would be effective only if well timed. On the other hand, impulse propagation is irrelevant in IL endocrine cells because of their compact morphology. Receptor-mediated control of release from endocrine cells results from a modulation of endogenous rhythmic electrical activity (Douglas, 1968; Davis and Hadley, 1978), and the slower kinetics in IL $GABA_A$ receptors may have an adaptive value for the regulation of oscillation frequency. These two situations place different demands on the biophysical properties of inhibitory receptors, and the IL and PP $GABA_A$ receptor characteristics described here may be tailored for specific physiological roles.

Multiple Affinities for DHEAS in PP and IL. Concentration-inhibition plots for both PP and IL $GABA_A$ receptors were better fitted by a two-binding-site model than by a one-binding-site model. In PP, blockade was dominated by a low-affinity component with an apparent dissociation constant of 15 μM. However, the PP may also have a high-affinity site because a small amount of blockade ($\sim$28%) could be seen with DHEAS in the nanomolar range. This may reflect an additional DHEAS binding site with weak negative control over receptor function. A high-affinity site could also be an indication of two receptor populations, as might be expected from the diversity of neuroactive steroid efficacies in a number of tissues (Gee et al., 1995; Nguyen et al., 1995).

DHEAS blockade also revealed two binding sites in IL, differing by $\sim$1000-fold in sensitivity. High variability in DHEAS sensitivity prompted us to divide IL cells into two groups. The segregation of this property between different IL cell populations suggests some form of structural distinction, such as subunit composition, receptor phosphorylation, associated proteins, or lipid environment. The most likely explanation is subunit composition, which has been shown to underlie much of the pharmacological diversity of $GABA_A$ receptors (Whiting et al., 1995). mRNAs encoding α2, α3, β1, β3, γ2s, and γ1 $GABA_A$ receptor subunits have been detected in the IL (Berman et al., 1994), and these subunits could be combined in many ways. The presence of β subunits is significant because receptors formed from a β subunit alone can be modulated by neuroactive steroids (Puia et al., 1990). However, other subunits are likely to be involved, and a more detailed understanding of the structural determinants of neuroactive steroid sensitivity will require further investigation (Whiting et al., 1995).

The two IL $GABA_A$ receptors generated responses with similar kinetic properties and sensitivities to GABA. The amplitudes and desensitization rates were indistinguishable (Table 1), as were the apparent dissociation constants and Hill coefficients for activation by GABA (Fig. 7). This suggests that the structural factors responsible for differences in DHEAS sensitivity are highly localized and specialized. It may just be a single subunit that is different between the two receptor forms. Thus, the structural elements that determine GABA sensitivity and desensitization kinetics could be the same in both high-sensitivity and low-sensitivity IL cells (but different in PP nerve terminals). The structural elements that determine DHEAS binding would then appear to have little influence over these other parameters. Once the molecular structures of these two closely related IL $GABA_A$ receptors are determined, sequence comparisons are likely to provide valuable clues about DHEAS binding domains.

DHEAS has previously been shown to act as a noncompetitive antagonist at $GABA_A$ receptors in binding assays and electrophysiological studies. In binding assays in neuronal synaptosomal membranes, a two-binding-site model fitted the data, yielding apparent dissociation constants of 2.9 and

554 μM (Majewska et al., 1990). Electrophysiological studies suggested blockade at a single binding site, with an IC_{50} value of 13 μM (Majewska et al., 1990) or 4.5 μM (Spivak, 1994). In another study, DHEAS was found to inhibit GABA-induced $^{36}Cl^-$ influx in cortical neurons with an IC_{50} value of 10 μM (Souza and Ticku, 1997). The low-affinity binding site seen in both IL and PP is consistent with these earlier studies, but to our knowledge a nanomolar sensitivity of $GABA_A$ receptors to DHEAS, as described here, has not been reported previously.

Physiological Roles for DHEAS Blockade of Pituitary $GABA_A$ Receptors. Because the neurointermediate lobe of the pituitary gland lies outside the blood-brain barrier, it will be exposed to circulating DHEAS. In humans, circulating DHEAS levels are generally in the micromolar range (Sulcová et al., 1997), but in rodents, they are only ~1 nM (Corpéchot et al., 1981). Thus, the high-sensitivity site described here in rat could place IL $GABA_A$ receptors under the regulatory control of circulating DHEAS. The low-sensitivity site would then be free to respond if DHEAS levels rise. The IL secretes several hormones derived from the proopiomelanocortin precursor, including α-melanocyte-stimulating hormone and β-endorphin. The variable sensitivity to DHEAS raises the interesting possibility of differential control of these different peptide hormones. Alternatively, the variable sensitivity may provide for graded control of each peptide hormone over a wide range of DHEAS levels.

Both the IL and PP receive GABAergic innervation from the hypothalamus (Oertel et al., 1982; Vincent et al., 1982), and it has been shown that GABA can either inhibit or enhance hormone release from the pituitary gland depending on the stimulation protocol (Tomiko et al., 1983; Fjalland et al., 1987; Saridaki et al., 1989). With the receptor sensitivities reported here, physiological levels of DHEAS would be capable of modulating $GABA_A$ receptors in both the IL and PP. Actions on these and other endocrine systems could be relevant to some of the physiological functions proposed for this neuroactive steroid (Baulieu and Robel, 1996; Bastianetto and Quirion, 1997).

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