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Potentiation of the depression by adenosine of rat cerebral cortical neurones by progestational agents

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1 The effects of four progestational agents pregnenolone sulphate, cyproterone acetate, norethindrone acetate and progesterone, on adenosine-evoked depression of the firing of rat cerebral cortical neurones have been studied.

2 When applied iontophoretically, pregnenolone sulphate, cyproterone, and norethindrone enhanced the actions of iontophoretically applied adenosine and failed to potentiate the depressant effects of adenosine 5'-N-ethylcarboxamide and γ-aminobutyric acid.

3 Cyproterone acetate ($50\,\mu g\,kg^{-1}$) and progesterone ($200\,\mu g\,kg^{-1}$) administered intravenously enhanced the depressant actions of iontophoretically applied adenosine.

4 When applied by large currents, cyproterone, and less frequently norethindrone, depressed the firing of cerebral cortical neurones. The depressant effects of cyproterone were antagonized by caffeine.

5 Pregnenolone sulphate tended to excite cortical neurones but neither this action, nor its potentiation of adenosine were reproduced by application of sulphate ions.

6 It is hypothesized that some of the psychotropic actions of progestational agents may involve an enhancement of 'purinergic' tone in the central nervous system.

Introduction

The relationship between steroid hormones and brain function has generally been considered from the point of view of feedback regulation of brain and anterior pituitary activity by the secretory products of endocrine glands. It is now accepted that steroid hormones exert this form of feedback control upon pituitary secretion of their respective trophic hormones by binding to intracellular receptors and altering gene transcription in target cells within specific neuroendocrine structures (McEwan & Parsons, 1982; Pfaff & McEwan, 1983).

Intravenously or iontophoretically administered steroids can also elicit very rapid changes in neuronal excitability, which are likely to be mediated by plasma membrane-bound receptors rather than by genomic mechanisms (Moss & Dudley, 1984). Although no interactions with a specific component of the nerve cell membrane have yet been documented, there are intriguing indications that glucocorticoids can affect responses to putative transmitters such as γ-aminobutyric acid (GABA), acetylcholine and noradrenaline (Janowsky & Davis, 1970; Simmonds *et al.*, 1984; Maggi & Perez, 1984; Majewska *et al.*, 1985; Kow & Pfaff, 1985).

There have been a number of studies of the actions of iontophoretically applied glucocorticoids with rapid depression of the firing of hypothalamic neurones being a frequent observation (Ruf & Steiner, 1967; Mandelbrod *et al.*, 1974; Kelly *et al.*, 1977; Steiner *et al.*, 1979). However, neurones in regions of the brain other than the hypothalamus have received less attention from steroid pharmacologists, for it is in the hypothalamus and some adjacent areas that many of the steroid hormone concentrating cells are observed (Warembourg, 1985).

Although the cerebral cortex does not contain neurones which concentrate progesterone and oestrogen, responses to these steroids have been observed following their intravenous or iontophoretic application. Progesterone induced rapid changes in the firing patterns of cerebral cortical neurones and depressed synaptic activity evoked by sensory stimuli (Komisaruk *et al.*, 1967). The frequency of interictal spikes recorded from a penicillin focus in the cat cerebral cortex was depressed by intravenously or locally administered progesterone (Landgren *et al.*, 1978).

A potential involvement of adenosine in this depressant action of progesterone was suggested by our observations that various steroids, including progesterone, were inhibitors of adenosine uptake by rat

brain cortical synaptosomes (Phillis & Wu, 1982; Phillis *et al.*, 1985). In related experiments, an ability of iontophoretically applied progesterone to potentiate the depressant actions of locally applied adenosine on rat cerebral cortical neurones was established (Phillis *et al.*, 1985) and it was suggested that enhancement of the actions of endogenously released adenosine could be involved in the known hypnotic, anticonvulsant, and analgesic actions of progesterone (Gyermek & Soyka, 1975). Because of difficulties stemming from the insolubility of progesterone in the aqueous solutions required for iontophoretic electrodes, it was decided to pursue these experiments with other, more soluble, progestational agents, including norethindrone acetate (Norlutate), cyproterone acetate (Androcur) and pregnenolone sulphate. Evidence for a specific action on the adenosine uptake system was obtained by comparing the effects of these progestins on adenosine, adenosine 5'-N-ethyl carboxamide (NECA) and GABA evoked depressions. NECA is an uptake-resistant analogue of adenosine (Wu *et al.*, 1984; Ehinger & Perez, 1984). Finally, intravenously administered progesterone and cyproterone acetate were observed to potentiate the depressant effects of iontophoretically applied adenosine.

Methods

The experiments described here were conducted on 41 adult male Sprague-Dawley rats (350–375 g body weight). The animals were anaesthetized with halothane, and after insertion of a tracheal cannula, anaesthesia was maintained with a mixture of methoxyflurane, nitrous oxide (75%), and oxygen (25%). The animals were placed in a stereotaxic frame and body temperature was maintained at 37°C via an electric heating pad controlled by a rectal thermal probe. After reflection of the skin overlying the dorsal skull, a small hole was drilled through the parietal bone 2 mm lateral to the sagittal suture and 1.5 mm posterior to the coronal suture line. This hole allowed access to the sensorimotor cortex after a slit had been made in the dura mater. The exposed skin, muscle, and bone were covered with a thin layer of 4% agar in Ringer solution to prevent drying and to stabilize the cortical surface. A venous cannula was placed in the right femoral vein.

Seven-barrelled micropipettes were used to record extracellular action potentials and apply drugs by iontophoresis or electro-osmosis onto cerebral cortical neurones. The central recording barrel and one side barrel were filled with 2 M NaCl and the remaining barrels were filled by centrifugation with various combinations of the following substances: adenosine hemisulphate (0.1 M, pH 4.0), acetylcholine chloride (0.1 M, pH 5.0), adenosine-5'-N-ethyl-carboxamide (0.01 M, pH 6.0, a gift of the Warner-Lambert Co.), γ-aminobutyric acid (GABA) (0.1 M, pH 4.5), cyproterone acetate (0.1 mM, in 50 mM NaCl, pH 3.8, a gift of Schering AG), norethindrone acetate (0.1 mM in 50 mM NaCl, pH 3.8, Sigma), pregnenolone sulphate, sodium salt (0.01 M, pH 7.8, Sigma), and sodium sulphate (0.01 M, pH 5.7). Cyproterone and norethindrone were ejected as cations; pregnenolone as an anion. Substances were tested on deep (1,000–1,400 μm), spontaneously firing, acetylcholine-excited neurones in the sensorimotor cortex. Earlier studies have shown that most of these neurones can be identified as corticospinal cells (Phillis *et al.*, 1979). Pregnenolone sulphate was tested on a number of antidromically identified corticospinal neurones. A small hole was made through the interparietal bone over the ipsilateral cerebellar cortex 0.5 mm lateral to the midline and 10.5 mm posterior to bregma. A bipolar coaxial stimulating electrode was placed in the ipsilateral pyramidal tract through this access hole. Neurones were identified as corticospinal cells if they responded to ipsilateral pyramidal tract stimulation with a short, constant-latency spike that followed stimulation frequencies of at least $100\,s^{-1}$. Steroid effects were evaluated by observing changes in the rate of spontaneous firing or by monitoring their effects on the depressant action of adenosine.

Adenosine was applied repetitively by 15–20 s pulses of current generated by a Dagan polarizer with automatic current balancing capability at interpulse intervals of 90–240 s. When the responses to adenosine had stabilized, steroids were applied concurrently for periods of up to 6 min. The durations of the depressions of spontaneous firing elicited by three presteroid applications of adenosine were measured and compared with those of three consecutive adenosine responses during and immediately following the application of steroid. Adenosine applications were continued until the duration of the depressions had recovered to control values. Similar experiments were conducted with NECA or GABA replacing adenosine as the inhibitory agent. Cyproterone can have a depressant action on the firing of cerebral cortical neurones. Therefore, in the present studies with adenosine, it was also tested in the absence of adenosine applications to ensure that the amounts applied were insufficient to depress neuronal firing.

In thirteen animals, cyproterone or progesterone were administered intravenously and their effects on the duration of iontophoretically applied adenosine-evoked depressions ascertained. For intravenous administration, cyproterone and progesterone were dissolved in small amounts of dimethyl sulphoxide (0.05 ml), which does not affect adenosine responses (Phillis, 1984). Student's *t* test for paired data was used to assess the statistical significance of the results.

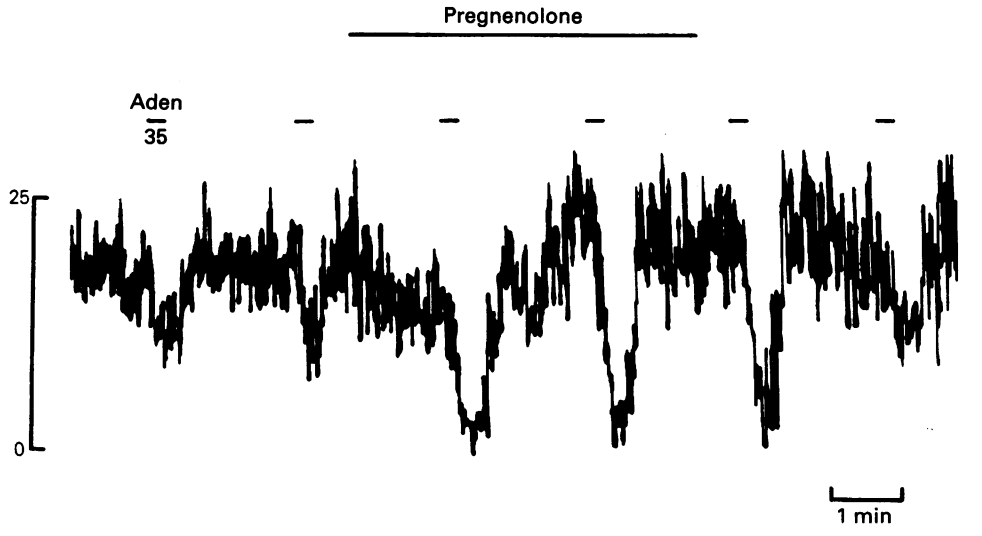

Figure 1 Firing frequency record of a spontaneously firing rat cerebral cortical neurone. This is a rate meter recording with the number of action potentials per second on the ordinate scale. Horizontal bars above the recordings indicate periods of drug application. Adenosine (Aden, 35 nA) reduced the rate of firing. During an application of pregnenolone sulphate (80 nA) the effects of adenosine were potentiated in magnitude and duration, even though the spontaneous firing rate had slightly increased. Return to control firing rates and of the adenosine responses to control amplitudes occurred in 5 min.

Results

Pregnenolone sulphate

The spontaneous activity of cerebral cortical neurones was recorded before, during and after the iontophoretic application of pregnenolone sulphate, with iontophoretic currents of 50–150 nA. Eighteen of the 108 neurones tested with this steroid exhibited an increase in their discharge frequency. No inhibitory effects were observed when pregnenolone sulphate was applied by itself. The latency of the excitatory response was found within a 15–45 s range and the excitation outlasted the period of steroid application. The studies of LaBella *et al.* (1979) have demonstrated that steroid sulphates were able to elicit brain responses that seemed to depend on the sulphate moiety of the molecule. However the excitations induced by pregnenolone sulphate were not reproduced by sulphate ions on the 24 neurones tested.

For tests of its interactions with adenosine-evoked depressions, the pregnenolone application currents were adjusted to have a minimal effect on spontaneous firing rates. Adenosine depresses the spontaneous firing of cerebral cortical neurones. Once the responses to adenosine had stabilized, pregnenolone was applied concurrently and the change in the magnitude and duration of the adenosine-evoked depressions observed. An example of the testing paradigm is illustrated in Figure 1. Adenosine (35 nA) depressed the spontaneous firing of this neurone. Pregnenolone was then applied for 5 min. During this period the responses to adenosine were enhanced in magnitude and duration even though the rate of spontaneous firing had increased slightly. Potentiation of the adenosine response was short-lived and partial recovery to control response level had occurred within 3 min. The effects of pregnenolone on adenosine responses were ascertained on 55 neurones of which 20 were identified as corticospinal cells by the invasion of an antidromic spike following pyramidal tract stimulation. The mean duration of control adenosine depressions was 64.9 ± 1.7 s. During and immediately following pregnenolone administration, the duration of adenosine depressions increased to 80.8 ± 1.8 s a 25% increase in duration ($P < 0.001$) (Table 1). Enhancement of the adenosine response was observed for every neurone, with the actual magnitude of increase in duration ranging between 2.5 and 82%. A feature of the potentiating action of pregnenolone was the relatively rapid time course of the effect. Potentiation of the adenosine response was generally apparent

Table 1 Potentiation of depressant effects of adenosine, but not of adenosine-5'-N-ethylcarboxamide (NECA), on rat cerebral control neurones by iontophoretically or intravenously administered progestins

Agent	*Mean duration of of adenosine depression*	*Mean duration of depressions with adenosine + agent*	*% change of duration of adenosine response*	*Mean duration of NECA depressions*	*Mean duration of depressions with NECA + agent*	*% change in duration of NECA response*
I. *Iontophoretic studies*						
Pregnenolone (sulphate)	64.9 ± 1.7 ($n = 55$)	80.8 ± 1.8***	+24	72.7 ± 2.2 ($n = 16$)	70.0 ± 2.1	−4
Cyproterone (acetate)	81.35 ± 2.6 ($n = 25$)	101.7 ± 2.2***	+25	96.2 ± 3.8 ($n = 16$)	89.1 ± 3.0	−7.4
Norethindrone (acetate)	42.6 ± 0.9 ($n = 49$)	52.2 ± 1.4***	+22.5	67.9 ± 2.8 ($n = 15$)	61.9 ± 3.1	−8.9
Sulphate (sodium)	56.7 ± 2.4 ($n = 13$)	50.2 ± 2.0	−11.5			
II *Systemic (i.v.) injection*						
Cyproterone acetate ($50\,\mu g\,kg^{-1}$)	67.8 ± 11.8 ($n = 6$)	109.3 ± 9.5*	+61			
Progesterone $200\,\mu g\,kg^{-1}$	91.8 ± 5.3 ($n = 7$)	143.6 ± 8.4**	+56			

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

within 1–2 min of the onset of application and recovery occurred within 3–5 min of its termination. No potentiation of the adenosine response was observed during the ejection of sulphate ions (Table 1).

As a control for the specificity of pregnenolone-elicited enhancement of adenosine responses, 16 cells were tested for interactions between pregnenolone and 5'-N-ethyl carboxamide adenosine, an adenosine analogue which is not an effective substrate for the adenosine transport system in brain cell membranes. In these experiments, pregnenolone, applied for periods of up to 6 min, failed to enhance the amplitude or duration (Table 1) of NECA-evoked depressions (control depression = 72.7 ± 2.2 s; with pregnenolone = 70.0 ± 2.1 s; $P > 0.1$).

As a further confirmation of the specificity of the interaction between pregnenolone and adenosine, it was tested in conjunction with GABA on 37 neurones (Table 2). An example of the recording from one such neurone is presented in Figure 2. A neurone that increased its rate of discharge during pregnenolone administration was deliberately used for the figure to display this phenomenon, as well as the failure of pregnenolone to potentiate GABA. During the application of pregnenolone, the mean duration of GABA-evoked inhibitions was reduced from 17.8 ± 0.5 s to 14.5 ± 0.7 s, a reduction of 18.6% ($P < 0.01$). The question of whether this reduction in the duration of the GABA responses, which was often associated with a small reduction in the magnitude of the inhibition, was a result of the weak excitant action of pregnenolone, or if it represents an actual antagonism of GABA action remains unresolved.

Cyproterone

Cyproterone-evoked depressions of spontaneous firing were observed with several cells, when large application currents (> 150 nA) were used. Such inhibitions were usually slow in onset, requiring one or more minutes of drug application and recovery occurred slowly over several minutes when the application was terminated.

Cyproterone, applied iontophoretically in amounts that did not directly affect spontaneous firing, was tested on 25 cortical neurones, and potentiated the duration of adenosine-evoked depressions of 23 of these (Table 1). The mean duration of the adenosine-evoked control depressions was 81.35 ± 2.6 s. During and immediately following the application of cyproterone, the mean duration of the adenosine response for all 25 neurones was increased to 101.7 ± 2.2 s, a 25% increase in duration ($P < 0.001$). The effects of cyproterone on adenosine persisted for 15–20 min after its application.

Cyproterone was administered intravenously to 6

Table 2 Lack of potentiation of GABA inhibitions of rat cerebral cortical neurones by progestins

Agent	*Mean duration of GABA depression*	*Mean duration of GABA + agent*	*% change of duration of GABA inhibition*
Pregnenolone (sulphate)	17.8 ± 0.5 ($n = 37$)	14.5 ± 0.7**	−18.6
Cyproterone (acetate)	23.0 ± 1.0 ($n = 15$)	21.4 ± 1.1	−7.0
Norethindrone (acetate)	24.4 ± 1.7 ($n = 15$)	25.2 ± 0.8	+3.3

** $P < 0.001$

rats in a dose of $50\,\mu g\,kg^{-1}$. In this amount, cyproterone did not affect the spontaneous firing rate, but did enhance adenosine. The mean duration for the adenosine controls of the 6 neurones tested was 67.8 ± 11.8 s. After cyproterone, the mean duration of the adenosine responses increased to 109.3 ± 9.6 s ($P < 0.05$). At a dose of $500\,\mu g\,kg^{-1}$, intravenously administered cyproterone depressed the spontaneous firing of 4 of the 6 cells tested. The duration of the depression ranged from 4–12 min.

As a control for the specificity of cyproterone-induced enhancement of adenosine responses, its effects on inhibition elicited by NECA or GABA were also ascertained. In these experiments, cyproterone failed to enhance the amplitude or duration of NECA-evoked inhibitions of 16 neurones (Table 2). On several of these neurones there was evidence for some reduction of NECA-evoked responses during the cyproterone application, even though the spontaneous firing rate was unaltered. This effect may account for the small decrease in the duration of NECA-evoked inhibitions.

A small decrease was also observed in the duration of GABA-evoked inhibition recorded from 15 cells during cyproterone application (Table 2).

Caffeine competitively antagonizes the depressant actions of adenosine on cerebral cortical neurones and it was therefore of interest to observe whether this methylxanthine could antagonize the depressant effects of iontophoretically applied cyproterone. In experiments conducted on 6 animals, caffeine (20 or $30\,mg\,kg^{-1}$) was administered intravenously after reproducible control inhibitions had been recorded during iontophoretic applications of cyproterone. In

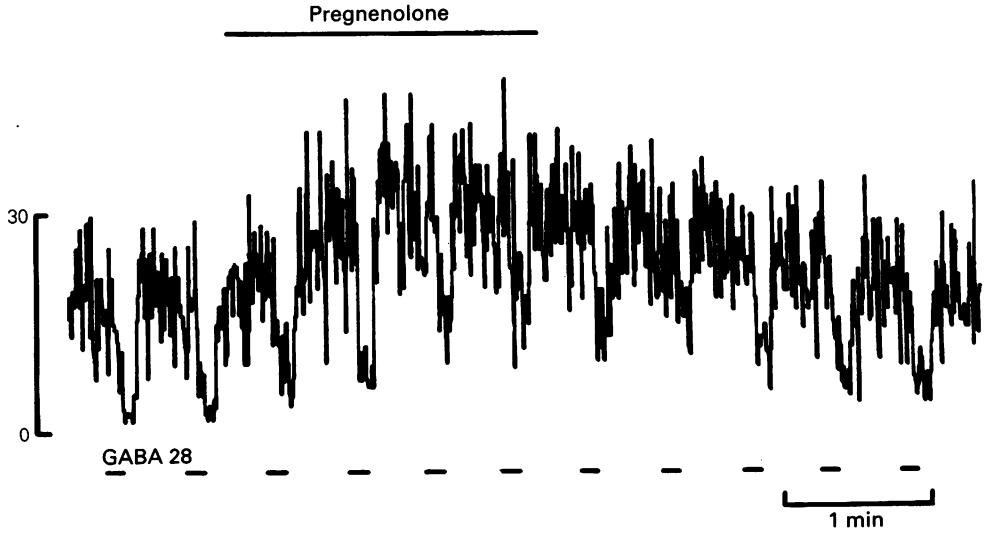

Figure 2 A rat cerebral cortical neurone which increased its rate of discharge during an application of pregnenolone sulphate (90 nA). The magnitude of GABA (28 nA)-evoked inhibitions of firing were unaltered by pregnenolone sulphate, whilst the duration of the inhibitions was slightly reduced.

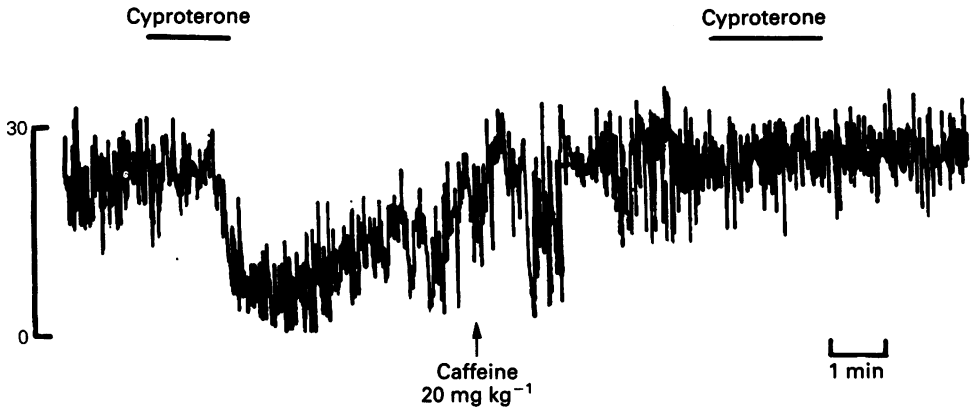

Figure 3 Cyproterone (120 nA) application resulted in a reduction in the firing rate of this cortical neurone. Recovery of firing to control levels was accelerated by the intravenous administration of caffeine (20 mg kg^{-1}). When tested after caffeine, a larger (120 s vs 90 s for control) application of cyproterone failed to elicit depression of firing. Partial recovery of the cyproterone response was evident 120 min after the caffeine dose.

each instance, the depressant effects of cyproterone were either greatly attenuated or abolished by the administration of caffeine (Figure 3). Partial recoveries from the antagonism by caffeine began to be apparent some 2 h after its administration.

Norethindrone

Norethindrone was tested on 79 neurones and was observed to depress the spontaneous firing of 12 of these. Unlike the depressant effects of cyproterone, those of norethindrone were often relatively rapid in onset (< 1 min) and recovery occurred within 2–3 min of the termination of application.

The interactions between adenosine and norethindrone were tested on 49 neurones, using norethindrone application currents which did not affect cell firing. Potentiation of the duration of adenosine responses was observed with 42 of these. The mean duration of the control response to adenosine was 42.6 ± 0.9 s (Table 1). During and immediately after norethindrone application the mean duration of purine-evoked responses recorded from all 49 neurones was 52.2 ± 1.4 s, a 22.5% increase

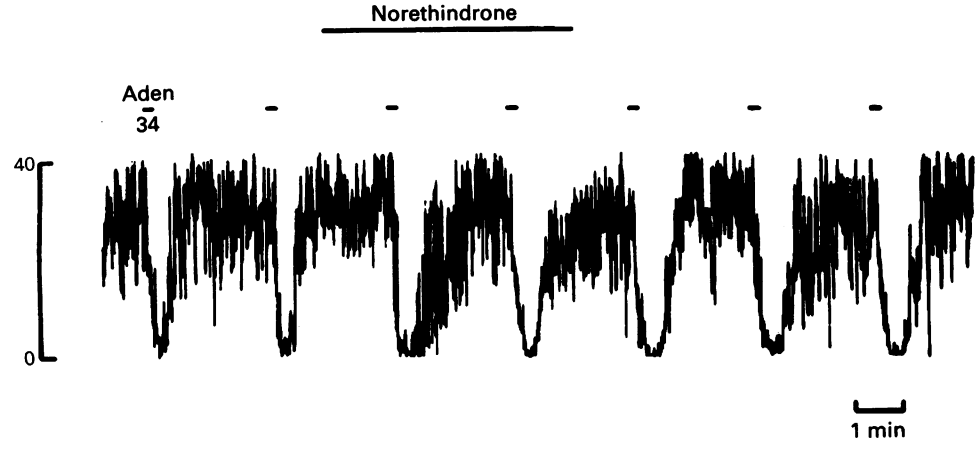

Figure 4 Potentiation of the depressant action of adenosine (Aden, 34 nA) on a rat cerebral cortical neurone by iontophoretically applied norethindrone (90 nA).

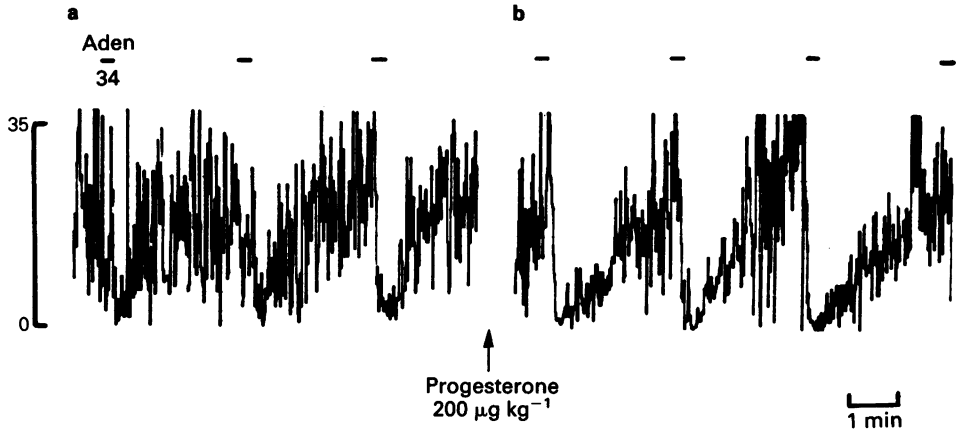

Figure 5 Potentiation of the depressant actions of iontophoretically applied adenosine (Aden, 44 nA) by intravenously administered progesterone (200 $\mu g\,kg^{-1}$). The post-progesterone responses were recorded 26 min after its administration.

($P < 0.001$). An example of norethindrone-evoked potentiation of adenosine depression is shown in Figure 4.

Norethindrone was tested with NECA on 15 cells, but failed to enhance the duration of the purine-evoked depressions (Table 1). Potential interactions between norethindrone and GABA were assessed on 15 cells. The mean duration of the GABA-induced inhibitions was 24.4 ± 1.7 s (Table 2). During the application of norethindrone, the mean duration of GABA inhibition was 25.2 ± 0.8 s, an increase of 3.3%.

Progesterone

Previous studies on the interaction between iontophoretically applied progesterone and adenosine (Phillis *et al.*, 1985) were complicated by the insolubility of progesterone in aqueous media and difficulties with the iontophoretic application of the steroid. In the current series of experiments progesterone was administered by intravenous application of a dose of 200 $\mu g\,kg^{-1}$.

The responses of 7 neurones to iontophoretically applied adenosine and the effects of progesterone on these responses were observed in the experiments. Spontaneous firing rates became slightly depressed within 5 min of progesterone administration in 5 of these trials, and returned to control levels within 20–70 min. Adenosine responses were measured when spontaneous firing rates had returned to control levels (Figure 5). Following progesterone administration the mean duration of adenosine-evoked depressions was prolonged from a control of 91.8 ± 5.3 s to 143.6 ± 8.4 s, an increase of 56% ($P < 0.01$). Return of the adenosine response duration to control levels was slow, with only partial recovery 2 h after progesterone administration.

Discussion

Our knowledge of the psychotropic effects of progestational agents presents a complex picture. It has been variously claimed that progestins in oral contraceptives are responsible for depression, lethargy and reduced libido (Kane *et al.*, 1967; Grant & Pryse-Davies, 1968) and that the luteal phase of the menstrual cycle is associated with reduced central activity (Moos, 1968; Bäckström *et al.*, 1983). Pregnancy, a period of high progesterone levels, is often a time of lowered incidence of emotional instability (Pugh *et al.*, 1963) and of euphoria (Dalton, 1964). It is also alleged that falling progesterone levels are involved in the aetiology of premenstrual tension (depression, tension, irritability, anxiety; Bäckström, 1977; Sanders *et al.*, 1983) and the instability of mood, anxiety, depression and insomnia near term and following pregnancy (Kane *et al.*, 1967; Treadway *et al.*, 1969). Changes in seizure frequency in epilepsy occurred during the menstrual cycle in some women: seizures were fewer during the luteal phase but increased when progesterone levels declined (catamenial epilepsy): these effects have been ascribed to a stabilizing action of progestins on central excitability (Bäckström, 1977; Newmark & Penry, 1980; Mattson & Cramer, 1985). Some improvement in seizure frequency control has been shown in pilot studies using medroxyprogesterone acetate (Mattson & Cramer, 1985).

In pharmacological doses, progesterone exhibits an

anaesthetic effect on rats (Selye, 1942). In addition progesterone has an anticonvulsant action (Spiegel & Wycis, 1945; Costa & Bonnycastle, 1952) and raises the threshold for electroshock seizure (Woolley & Timiras, 1962). A depressant effect of progesterone, in physiological amounts, on the generation of spikes from a penicillin focus in the cat cerebral cortex has been reported (Landgren *et al.*, 1978). Progesterone also depressed single unit responses in the rat cerebral cortex (Komisaruk *et al.*, 1967).

In a study of male rat cerebral cortical synaptosomes, it became apparent that progesterone is a potent inhibitor of adenosine uptake with IC_{20} and IC_{50} values of 1.1×10^{-8} M and 7.5×10^{-7} M (Phillis *et al.*, 1985). Further experiments on cultured neurones and astrocytes, have confirmed our earlier report of the adenosine-uptake blocking ability of progesterone. Adenosine uptake into cultured mouse neopallial astrocytes was inhibited by progesterone with an IC_{50} or 5.03 μM; that into primary cultures of mouse cerebral cortical neurones with an IC_{50} of 10.2 μM (A.S. Bender, personal communication). Studies on central neurones have shown that recognized uptake inhibitors, such as dilazep, lidoflazine and papaverine, have similar effects to progesterone in that they potentiate the duration of action of iontophoretically applied adenosine and can depress the firing of cortical neurones (Phillis *et al.*, 1979; Phillis & Wu, 1981), presumably by enhancing the extracellular concentrations of adenosine. Uptake inhibitors, administered intracerebroventricularly, depress locomotor activity in mice in a caffeine-sensitive fashion (Phillis *et al.*, 1986).

Progesterone concentrations in the plasma of female rats range between 2 ± 1 ng ml^{-1} and 46.7 ng ml^{-1} (10^{-8}–1.5×10^{-7} M) at various stages of the oestrous cycle (Butcher *et al.*, 1974). Plasma concentrations in human females can reach levels of 18 ng ml^{-1} (5.7×10^{-8} M) during the luteal phase of the menstrual cycle and in excess of 600 ng ml^{-1} during pregnancy (Bäckström, 1977). Progesterone accumulates in several regions of the central nervous system, including the cerebral cortex (Wade *et al.*, 1973; Bäckström, 1977) where concentrations can be fourfold those in the plasma.

The concentrations of progesterone in plasma and brain would therefore be sufficient, during periods of enhanced progesterone secretion in the luteal phase of the menstrual cycle, to cause a 25–40% inhibition of adenosine uptake into cerebral cortical nerve endings. As experiments on cardiac tissue have demonstrated that a 20% inhibition of the uptake system is sufficient to double the actions of exogenously applied adenosine (Hopkins, 1973), inhibition of adenosine uptake is likely to be a significant factor in the actions of endogenously released progesterone. This conclusion is supported by observations made in the present experiments where administration of a 200 μg kg^{-1} dose of progesterone itself, depressed cortical neuronal firing and prolonged the duration of adenosine-evoked depressions by 56%.

Pregnenolone and its sulphate ester have been characterized in the brain of adult male rats (Corpechot *et al.*, 1983). Both occurred in the forebrain in concentrations (38.4 ± 6.9 and 15.8 ± 3.0 ng g^{-1}) that largely exceeded those in the plasma (1.3 ± 0.2 and 1.4 ± 0.3 ng g^{-1}), liver, kidney, spleen and testes. Pregnenolone sulphate reportedly does not interact with the cytoplasmic receptors for steroids described in the Introduction (Robel & Baulieu, 1985) and its effects can therefore be perceived as occurring at the plasma membrane. Carette & Poulain (1984) applied pregnenolone sulphate by iontophoretic ejection onto neurones in the septo-preoptic area and observed an excitatory effect on some neurones (58%). Weak excitant effects were also observed in 17% of the neurones tested in the present series.

Iontophoretically applied pregnenolone sulphate significantly enhanced the duration of adenosine-evoked depressions of cerebral cortical neurones. The specificity of this potentiation was demonstrated in two tests. Pregnenolone sulphate did not potentiate the depressant actions of adenosine 5'-N-ethyl carboxamide, an uptake-resistant analogue of adenosine, nor did it potentiate the depressant action of GABA.

Cyproterone acetate applied either iontophoretically or by intravenous injection, potentiated the depressant actions of locally applied adenosine. In larger amounts, cyproterone depressed the firing of cortical neurones, an effect which was prevented by the adenosine antagonist, caffeine. The failure of cyproterone acetate to potentiate the depressant actions of an uptake resistant analogue of adenosine, NECA, implies that its effects were a result of the inhibition of adenosine uptake. It also failed to enhance the inhibitory actions of GABA. Cyproterone acetate, an antiandrogen with strong progestational properties has been used in patients suffering from pathological sexual hyperactivity. Amongst its effects, a decrease in energy and drive and sedation were noted (Itil *et al.*, 1974) and in clinical pilot trials cyproterone acetate was observed to have anxiolytic activity (Herrmann & Beach, 1978).

Norethindrone acetate is another potent progestin, with a demonstrable ability to inhibit adenosine uptake by central synaptomsomes (Phillis *et al.*, 1985). In accordance with the findings made with the other progestins, norethindrone acetate was observed to potentiate the depressant actions of adenosine, but not of NECA or GABA, and it had depressant effects on the firing of some neurones. Norethindrone is widely used in sequential type oral contraceptive preparations and its central actions may be responsible for some of the emotional changes that have been noted in

individuals using this form of contraception.

In conclusion, the findings presented in this paper demonstrate an ability of progestational agents to potentiate the actions of adenosine and argue for a potential involvement of adenosine in the emotional and neuropharmacological consequences of changes in progesterone levels. In particular, some of the emotional changes associated with the menstrual cycle and pregnancy may result from alterations in neuronal excitability due to fluctuations in the level of purinergic tone in the central nervous system. A reduction in purinergic inhibitory modulation of synaptic transmission, resulting from rapidly declining progesterone levels, may be a contributing factor in premenstrual tension and catamenial epilepsy.

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