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Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it

(memory enhancement/pregnenolone sulfate/receptors/immediate-early genes/aging)

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ABSTRACT **Immediate post-training intracerebroventricular administration to male mice of pregnenolone (P), pregnenolone sulfate (PS), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, testosterone, dihydrotestosterone, or aldosterone caused improvement of retention for footshock active avoidance training, while estrone, estradiol, progesterone, or 16β-bromoepiandrosterone did not. Dose–response curves were obtained for P, PS, DHEA, and testosterone. P and PS were the most potent, PS showing significant effects at 3.5 fmol per mouse. The active steroids did not show discernible structural features or known membrane or biochemical effects that correlated with their memory-enhancing capacity. The above, together with the findings that DHEA acted even when given at 1 hr after training and that P, PS, and DHEA improved retention over a much wider dose range than do excitatory memory enhancers, led to the suggestion that the effects of the active steroids converge at the facilitation of transcription of immediate-early genes. P and PS, for which receptors have not yet been demonstrated, may exert their effects by serving as precursors for the formation of a panoply of different steroids, ensuring near-optimal modulation of transcription of immediate-early genes required for achieving the plastic changes of memory processes. Low serum levels of P in aging and the increases of cancer and behavioral disorders in individuals receiving drugs that block synthesis of cholesterol, the immediate precursor of P, suggest possible clinical utility for P.**

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS), major circulating steroids in humans, are largely adrenally derived substances which serve as precursors for both androgenic and estrogenic steroids (1). These substances appear to play important roles in many aspects of cellular controls (1–5). Although molecular mechanisms of effects in particular instances have not yet been defined, the overall experimental results may be considered to be examples of pleiotropic facilitation of coordinative processes that enable immune, neural, and metabolic systems, separately and together, to cycle freely through their operational modes in solving problems of survival and reproduction and in achieving rebalancing when malfunctioning occurs (4, 5).

Blood levels of DHEA and DHEAS are reduced in aging (6) and during prolonged acute stress (1). We tested the supposition (5) that raising extracellular levels of DHEA, DHEAS, or both might have some beneficial effects on aspects of nervous system function. Low concentrations of DHEA and DHEAS reduced neuronal death and enhanced astrocytic differentiation while decreasing proliferation of astrocytes in brain cell culture. Expression of cellular properties related to postmitotic differentiated states was favored in both neurons and glia (7, 8).

The water-soluble DHEAS, administered intracerebroventricularly (i.c.v.) or subcutaneously (s.c.) after training, showed convincing memory-enhancing (ME) effects in footshock active avoidance training (FAAT) in undertrained male mice. DHEAS administered i.c.v. facilitated retention for step-down passive avoidance training. *Retention* of FAAT was improved when DHEAS was given in taste-camouflaged drinking water for 1 week before and 1 week after training, while DHEAS in the drinking water for 2 weeks did not improve *acquisition* (9). DHEAS also completely counteracted the amnestic effects of anisomycin and scopolamine (9). A single s.c. injection of DHEAS after training improved defective memory processes in aging mice, returning retention of FAAT to the high levels observed in young mice (10). DHEA is insoluble in water but freely soluble in dimethyl sulfoxide (DMSO). Pure DMSO (2 μl) administered i.c.v. caused amnesia. DHEA in DMSO (i.c.v., after training) was active in impressively low amounts (3.5 pmol per mouse) in significantly improving retention relative to control groups receiving DMSO alone (9). Thus, inadequate availability of DHEA, DHEAS, or steroids derived from them metabolically could be rate-limiting in achievement of plastic changes required for retention of learning to take place.

The results cited above and those in the literature were compatible with the idea that these substances might be helpful in treatment of neurological diseases associated with aging and metabolic or immune dysregulation. Clinical tests with oral DHEA have been initiated in patients with multiple sclerosis (11), Alzheimer disease (12), and benign memory deficit.

Cortisol, corticosterone, and dexamethasone previously had been shown to improve retention of FAAT (13).

MATERIALS AND METHODS

Steroids Tested. The following substances obtained from the indicated suppliers were employed in the tests to be described: DHEA from Searle; estradiol, estrone, testosterone, dihydrotestosterone, androstenedione, 17α-hydroxypregnenolone, and pregnenolone sulfate (PS) from Steraloids (Wilton, NH); aldosterone, pregnenolone (P), proges-

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Abbreviations: FAAT, footshock active avoidance training; i.c.v., intracerebroventricular(ly); s.c., subcutaneous(ly); DMSO, dimethyl sulfoxide; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; P, pregnenolone; PS, pregnenolone sulfate; IEG, immediate-early gene; ME, memory enhancement or memory-enhancing.

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Table 1. Effects of 350 pmol of i.c.v.-administered steroids on retention of T-maze FAAT

Substance tested	Trials to criterion*, no.	P value†	Substance tested	Trials to criterion*, no	P value†
Saline	6.80±0.25		Dexamethasone (1,4-Pregnadiene-9α-fluoro-16α-methyl-11β,17α,21-triol-3,20-dione)	¶	
DMSO	9.67±0.33‡				
Pregnenolone (5-Pregnen-3β-ol-20-one)	7.40±0.40	<0.01	Dehydroepiadrosterone (5-Androsten-3β-ol-17-one)	6.93±0.26	<0.01
Pregnenolone sulfate (5-Pregnen-3β-ol-20-one sulfate)	§		16α-Bromoepiandrosterone (5α-Androstane-16α-bromo-3β-ol-17-one)	9.08±0.59	NS
17α-Hydroxypregnenolone (5-Pregnene-3β,17α-diol-20-one)	7.27±0.37	<0.01	Dehydroepiandrosterone sulfate (5-Androsten-3β-ol-17-one sulfate)	‖	
Progesterone (4-Pregnene-3,20-dione)	8.93±0.37	NS	Androstenedione (4-Androstene-3,17-dione)	6.93±0.37	<0.01
Corticosterone (4-Pregnene-11β,21-diol-3,20-trione)	¶		Testosterone (4-Androsten-17β-ol-3-one)	6.67±0.28	<0.01
Aldosterone (4-Pregnene-11β,21-diol-3,18,20-trione)	7.17±0.28	<0.01	Dihydotestosterone (5α-Androstan-17β-ol-3-one)	7.20±0.44	<0.01
			17β-Estradiol (1,3,5(10)-Estratriene-3,17β-diol)	9.35±0.43	NS
Cortisol (4-Pregnene-11β,17α,21-triol-3,20-dione)	¶		Estrone (1,3,5(10)-Estratrien-3-ol-17-one)	9.20±0.42	NS

*Data for trials to criterion are expressed as mean ± SEM.
†*P* values for comparison with DMSO. NS, not significant.
‡*P* value for comparison with saline is <0.01.
§A memory enhancer (see Fig. 2).
¶Previously shown to be memory enhancers in mice by the s.c. route, with dexamethasone being the most potent (13).
‖Previously shown to be a memory enhancer in mice by i.c.v., s.c., and oral routes (9).

terone, and DMSO from Sigma. We received 16α-bromoepiandrosterone from Paul Talalay (Johns Hopkins University School of Medicine, Baltimore).

With the exception of PS, all of the substances were dissolved in appropriate amounts in pure DMSO; 2 μl of DMSO alone or containing test substance was injected i.c.v. into each mouse within 2–3 min after training. PS was dissolved in 10 μl of DMSO and diluted with physiological saline to a final concentration of 0.001% DMSO or less in saline, and the results of injections of 2 μl of solutions containing PS were compared with those obtained with 2 μl of 0.001% DMSO in saline, which gave the same results as saline alone.

Test Animals, Apparatus, and Training and Testing Procedures. The T-maze was used for FAAT and mice were prepared for i.c.v. injection as previously described (9). Male mice were assigned randomly to groups of 15 in the experiments reported in Table 1 and groups of 10 for the dose–response curves (Figs. 1 and 2) and were given training trials under appropriate conditions (see below). One week after training and post-trial i.c.v. administration of vehicle alone or vehicle containing test substance, retention was tested by continuing T-maze training until each mouse made five avoidance responses in six consecutive training trials (trials to criterion). Well-trained animals (mean of about seven trials to criterion) were used to determine whether water-insoluble

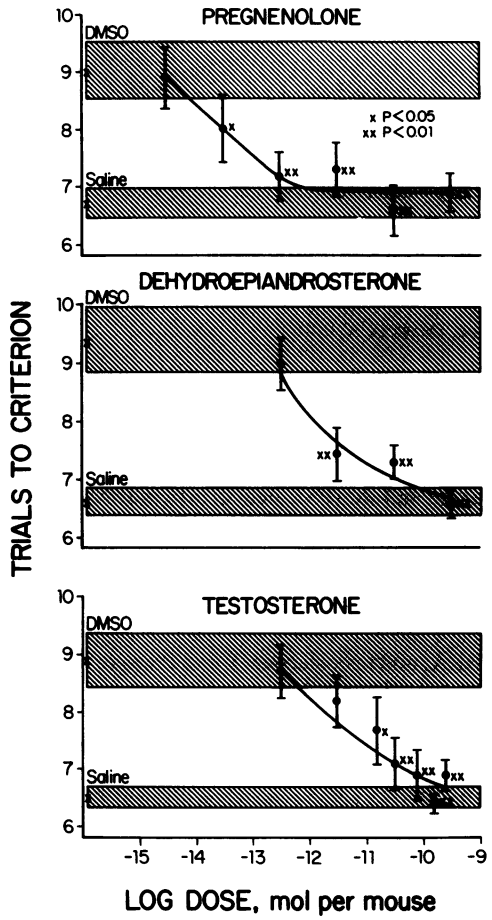

FIG. 1. Dose–response curves showing ME effects of P, DHEA, and testosterone on retention of T-maze FAAT measured in well-trained animals as the extent of prevention by substances dissolved in DMSO of the amnesia produced by pure DMSO alone. Data for trials to criterion are expressed as mean ± SEM.

substances dissolved in DMSO could prevent amnesia induced by DMSO alone. In these instances, training was performed under conditions that tend to maximize learning (five training trials; sound intensity, 65 decibels; footshock current, 0.35 mA; intertrial interval, 45 s). To determine whether the water-soluble PS could enhance memory processing, training conditions were adjusted so that vehicle controls (0.001% DMSO in saline) had poor retention, with a mean of about 10 trials to criterion (four training trials; sound intensity, 55 decibels; footshock current, 0.30 mA; intertrial interval, 30 s).

Statistical Treatment of Data. Results were expressed in terms of the mean and SEM. Significance of overall effects of treatment was determined by one-way analysis of variance run on trials to criterion from the retention test. Dunnett's *t* test was used to make multiple comparisons of individual test groups with control groups, and statistical comparisons among experimental groups were made by Tukey's *t* test (14–16).

RESULTS

The experiments in Table 1 tested whether or not a particular steroid showed memory enhancement (ME) at the dose of 350 pmol per mouse. Although DHEA was active at 3.5 pmol per mouse (9), a higher dose was chosen so that substances with weaker effects might be detected.

Mice were injected 2–3 min after FAAT with 350 pmol of the steroids indicated in Table 1 or with vehicle alone (DMSO). Testing for retention of learning took place 1 week

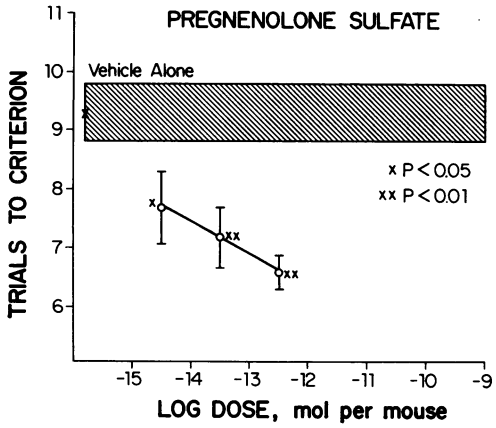

FIG. 2. Dose–response curve showing ME effect of PS on retention of T-maze FAAT measured in weakly trained animals as the extent of enhancement of retention by PS over that found with vehicle alone (0.001% DMSO in saline). Data for trials to criterion are expressed as mean ± SEM.

later. There was a significant overall treatment effect ($F_{11.168} = 11.33$, $P < 0.01$). Trials to criterion for the DMSO-treated group were significantly greater than for the group receiving saline alone—i.e., DMSO impaired memory processing. The groups receiving P, DHEA, DHEAS, androstenedione, testosterone, dihydrotestosterone, or aldosterone showed significantly fewer trials to criterion than mice receiving DMSO alone. The groups receiving progesterone, estrone, estradiol, or 16β-bromoepiandrosterone were not significantly different from those with DMSO alone.

Dose–response curves (Fig. 1) were obtained for P and for two substances in the androgenic metabolic sequence deriving from P, DHEA and testosterone. P showed the most potent ME of the substances tested. There was a significant overall treatment effect with P ($F_{7.72} = 5.18$, $P < 0.001$). Statistically significant ME was found at 3.5×10^{-14} mol per mouse ($P < 0.05$), and values virtually identical with those for the saline-injected animals were achieved at 3.5×10^{-13} mol per mouse and at higher doses. Significant overall ME was obtained with DHEA ($F_{5.54} = 11.23$; $P < 0.001$) and testosterone ($F_{8.81} = 6.81$; $P < 0.001$) at individual doses beginning at 3.5×10^{-12} and 1.75×10^{-11} mol per mouse ($P < 0.05$ or less), respectively.

PS, like P, potently improved retention of FAAT. Weakly trained mice receiving only 3.5×10^{-15} mol per mouse required significantly fewer trials to criterion than did the vehicle controls (Fig. 2).

DISCUSSION

(i) ME steroids do not share discernible structural features or known membrane or biochemical effects that correlate closely with ME. Steroids of diverse structure and function showed ME (Table 1 and Fig. 3). No molecular feature was identified that distinguishes positive from negative compounds. Among the ME substances are those classically associated with different overall physiological mechanisms and that bind preferentially to different receptor proteins in target tissues (17). There also appears to be no common pattern of direct membrane effects. For example, DHEA, DHEAS, and PS, all showing ME, had excitatory (depolarizing) membrane effects when applied to neurons in the septopreoptic area of the guinea pig (18). PS is both a negative allosteric modulator of the γ-aminobutyric acid type A ($GABA_A$) inhibitory receptor complex (19–22) and a positive modulator of the *N*-methyl-D-aspartate excitatory receptor complex (23). However, 17β-estradiol, negative in our study,

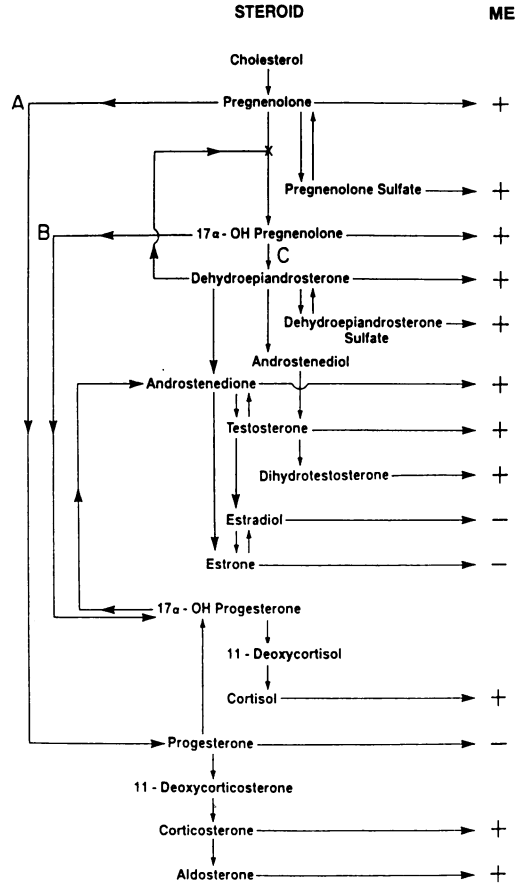

FIG. 3. Partial outline of the steroid metabolic scheme (1) as it may occur in an organism as a whole; relationships of substances tested for ME on retention of FAAT are shown. The biosynthesis of steroid hormones begins with synthesis of P from cholesterol, from which the sex steroids, glucocorticoids, and mineralocorticoids all eventually derive. P can be converted directly to progesterone and thence to aldosterone (route A) or to 17α-hydroxypregnenolone, which is a precursor for cortisol formation (route B) and for sex-related steroids (route C). Route A can contribute to route B and route B to route C, as shown. DHEA, the first product in route C, may inhibit the flow through routes B and C by inhibiting conversion of P to 17α-hydroxypregnenolone (1). PS and DHEAS can be formed from P and DHEA, respectively, by sulfotransferases and reconverted to the parent substances by sulfatases. If one were to homogenize an entire mammalian organism and extract the homogenate appropriately, one could expect to be able to find the steroids shown (and many others) and to demonstrate activities of enzymes that catalyze the indicated interconversions. However, it is highly unlikely that any single tissue contains all of the substances and/or enzymes; among those tissues in which they do exist, marked differences in levels would be found. Probably all cells in the body require for regulation of their functions some or all of these steroids, whose pleiotropic activities range from modulation of membrane excitability to regulation of genomic transcription.

had an excitatory effect on pyramidal neurons of rat hippocampus (24) and augmented cerebellar Purkinje cell responsivity to microiontophoretically applied glutamate (25). Cortisol, a memory enhancer (13), inhibited (hyperpolarized) neurons in guinea pig celiac ganglia (26). Moreover, the concentrations of PS required to exert effects on the $GABA_A$ receptor complex were in the micromolar range (20, 21), while significant ME (Fig. 2) was obtained with 1.75 nM (3.5 fmol/2 μl).

Effects of DHEA in various test systems have been attributed to its noncompetitive inhibition of mammalian glucose-6-phosphate dehydrogenase (G6PDH) (2, 27). However, 16α-bromoepiandrosterone, which is 60 times more potent than

DHEA as an inhibitor of G6PDH (28), did not enhance memory (Table 1). It is, therefore, unlikely that ME by DHEA or other steroids can be attributed to inhibition of G6PDH.

(ii) Dose–response curves for the ME steroids are different from those usually obtained with excitatory substances. In the effective dose range, progressive increases of excitatory substances first increase responses to a maximum, beyond which decreasing responses are observed until a level is reached at which no significant effects are seen over the controls (29). The decrements observed at higher doses possibly may be attributable to incoordinations induced in management of intracellular free Ca^{2+}, leading to disorganization of the orderly release of reaction cascades (30, 31).

In our extensive experience, the inverted U-shaped dose–response curve described above usually covered a 2- to 5-fold dose range. However, we found previously (9) and confirmed presently (Fig. 1) that DHEA improved retention over a much wider range of doses (100-fold). P was effective over an even wider dose range (10,000-fold). PS was effective over the 100-fold range tested (Fig. 2); higher doses were not tested because results at the lower doses showed PS to be at least as effective as P. If these results should generalize to the human situation, the effects of variability in individual responsivity would be reduced greatly with DHEA, P, and PS, thus avoiding necessity for individual dose titration. Alone or in conjunction with other treatments, they might be more effective in treatment of memory deficits than substances having narrower therapeutic windows.

The results cited above differentiate the steroid effects from those of excitatory substances and suggest that the action of the steroids with ME occurs at processes beyond the point of membrane excitation. Consideration of current knowledge of action of these substances does not offer strong support for the possibility that direct effects on cGMP and cAMP metabolism are involved.

(iii) The steroids that improve memory retention when given long after fast neural events have ceased may act by modulating rates and amounts of transcription of immediate-early genes (IEGs). Amenability of memorial processes to enhancement long after the fast neural events during learning have occurred is not attributable to perseverative neuronal activity consisting of reverberation of impulses in neuronal circuits that outlasts the stimulating events (32). Substances that improve retention when given after training decline in effectiveness at progressively longer times after training, usually becoming ineffective at 60–90 min. For example, when mice were trained under conditions giving poor retention in saline-injected controls, i.c.v. administration of DHEAS in saline at 2, 30, or 60 min after training gave significant improvement (9). When DHEAS was injected 90 and 120 min after training, no enhancement of retention occurred. Intense membrane depolarization produced in rats by the convulsants Metrazole and picrotoxin resulted in various brain regions in rapid transient increases in mRNAs of several putative transcription factor genes (33), reaching maximal levels within 60 min but being barely detectable at 120 min. c-Fos mRNA reached a maximum in the brains of mice 60 min after injection of Metrazole and declined to basal levels within 180 min (34). Interdigitation of membrane and genomic functions also occurs during normal nerve activity (35, 36). Alterations in expression of IEGs occur in the mammalian suprachiasmatic nucleus of the hypothalamus in response to retinal illumination at times in the circadian cycle when light is capable of influencing entrainment (37). From the above, it is reasonable to suggest that DHEAS, which shows ME when given within approximately 1 hr after training (9), and probably other ME steroids, facilitates the transcription of IEGs that is initiated by adequate neural stimulation and that a consequence of this facilitation is the amplification of translational processes that eventually lead to the synthesis of enzymes, structural proteins, or both. Inhibitors of protein synthesis cause amnesia (38). Although inhibition of mRNA production also probably would be amnestic, problems of toxicity generally have made definitive experiments difficult to perform.

There is extensive overlap in binding site competition among substances that bind to various steroid receptors as well as "promiscuity" in binding of different steroid–receptor complexes to the same DNA sequence (17, 39–43). Modulation of some genes (e.g., IEGs) by steroids may involve combinatorial interactions. A continuum of binding specificities may exist from virtually complete "promiscuity," such as is found in the induction of the mammary tumor virus (43), to the extreme exclusivity of the induction of some secondary sex characteristics. High degree of specificity of binding of a steroid–receptor complex to a DNA region near a particular gene might be conferred by the nearby binding in target tissues of other transcriptional regulatory proteins.

(iv) P and PS, potent memory enhancers (Figs. 1 and 2) for which receptors have not yet been demonstrated, may exert their effects by serving as precursors for the formation of a panoply of different steroids (Fig. 3) during nervous system activity, thus ensuring near-optimal modulation of transcription of IEGs required for facilitation of the plastic changes in memory processes. P and PS, major steroidal constituents of mammalian brain, are formed in oligodendrocytes and probably also in astrocytes (44–48) from cholesterol by the side-chain cleaving enzyme, cytochrome $P\text{-}450_{scc}$, independently of peripheral sources. High levels of P, PS, DHEA, and DHEAS were found in cranial nerves and in various regions of human brain (49). Upon interacting with appropriate receptors, the above substances themselves, or other steroids formed from them, may play important gene-regulatory roles both in the glial cells in which they are formed and in other cells in the vicinity. Even in the resting state, neurons and glial, endothelial, ependymal, and other nonneural cells participate in many interactions that range from physical forces they exert on each other to exchanges of varieties of trophic and/or inhibitory substances (50). These interactions are greatly accelerated by nerve activity, during which all relevant cellular components could derive from glial sources a supply of P and PS from which to form the smorgasbord of steroids they require optimally to modulate the extent of genomic transcription needed for an adaptive response to take place.

Based on findings with P-binding proteins (BP) in brain (51) and adrenal cortex (52), the following speculative scenario can be proposed (52): Whether formed indigenously or externally derived, P binds to the phosphorylated form of the BP to form a P–BP complex. P has a much higher affinity for BP than it has for its metabolizing enzymes and, therefore, is stabilized in the complex. P–BP is transported to specific cytoplasmic (e.g., endoplasmic reticulum) and nuclear sites where it is destabilized by dephosphorylation. The P–BP complex dissociates and the P released is converted by local enzymes to other steroids, which in turn bind to specific receptors that become transcriptionally active or that may take part in nongenomic processes.

Steroids for which there are no binding proteins would be rapidly metabolized upon entering a cell, perhaps only a small fraction of the original substance being available for formation of transcriptionally active steroid–receptor complexes or for other processes. PS would be an exception, because it can be converted directly to P by sulfatase action. The greater ME shown by P and PS than by DHEA and testosterone (Figs. 1 and 2) might be attributed to more efficient intracellular processing of P, as suggested above, and to the formation from P of a more versatile mixture of steroids than from

any of the substances below it in the steroid metabolic hierarchy (Fig. 3).

(v) If P, PS, and steroids derived from them should become inadequately available, as during aging, stress, or disease, degenerative processes may be initiated upon occurrence of functional demands that require enhanced metabolic responses and genomic transcription to take place. Degenerative processes may not become irreversible as long as affected cells still possess receptors for the substances in question. Restoring adequate levels of rate-limiting steroids might permit a measure of recovery to take place. In this regard, it is of interest that greatly reduced serum levels of P and 17α-hydroxypregnenolone were found in aged patients with Alzheimer disease (12), the decreased levels probably being typical of aging nondemented patients and not specific for Alzheimer disease. Further study will reveal whether these results are indicative of an embarrassment of P synthesis and, if so, at which locus the rate limitation occurs.

If the synthesis of P were to become limiting in aging and in other conditions, it would be expected that inhibition of synthesis of its sole precursor, cholesterol (Fig. 3), would further compromise the overall steroid economy and that individuals to whom are given drugs that block cholesterol synthesis would show adverse effects. Indeed, studies have shown that such treatments, while ameliorating cardiac symptoms, actually have resulted in increases in noncardiac deaths, including cancer and nervous system-related phenomena such as accidents, suicides, and violence (53). Perhaps administration of P together with cholesterol-lowering drugs would ameliorate the above undesirable effects. Detailed evaluation of steroid metabolism in patients receiving such drugs is recommended.

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1. Parker, L. N. (1989) *Adrenal Androgens in Clinical Medicine* (Academic, New York).
2. Sonka, J. (1976) *Acta Univ. Carol Med.* **71**, 1–171.
3. Regelson, W., Loria, R. & Kalimi, M. (1988) *Ann. N.Y. Acad. Sci.* **521**, 260–273.
4. Roberts, E. (1990) *Prog. Brain Res.* **86**, 339–355.
5. Roberts, E. (1986) in *Treatment Development Strategies for Alzheimer's Disease*, eds. Crook, T., Bartus, R. T., Ferris, S. & Gershon, S. (Powley, Madison, CT), pp. 173–219.
6. Orentreich, N., Brind, J. L., Rizer, R. L. & Vogelman, J. H. (1984) *J. Clin. Endocrinol. Metab.* **59**, 551–555.
7. Roberts, E., Bologa, L., Flood, J. F. & Smith, G. E. (1987) *Brain Res.* **406**, 357–362.
8. Bologa, L., Sharma, J. & Roberts, E. (1987) *J. Neurosci. Res.* **17**, 225–234.
9. Flood, J. F., Smith, G. E. & Roberts, E. (1988) *Brain Res.* **447**, 269–278.
10. Flood, J. F. & Roberts, E. (1988) *Brain Res.* **448**, 178–181.
11. Roberts, E. & Fauble, T. J. (1990) in *The Biological Role of Dehydroepiandrosterone (DHEA)*, eds. Kalimi, M. & Regelson, W. (de Gruyter, Berlin), pp. 81–93.
12. Roberts, E. & Fitten, L. J. (1990) in *The Biological Role of Dehydroepiandrosterone (DHEA)*, eds. Kalimi, M. & Regelson, W. (de Gruyter, Berlin), pp. 43–63.
13. Flood, J. F., Vidal, D., Bennett, E. L., Orme, A. E., Vasquez, S. & Jarvik, M. E. (1978) *Pharmacol. Biochem. Behav.* **8**, 81–87.
14. Bruning, J. L. & Kintz, B. L. (1987) *Computational Handbook of Statistics* (Scott, Foresman, Glenview, IL), 3rd Ed., pp. 130–133.
15. Keppel, G. (1982) *Design and Analysis: A Researcher's Handbook* (Prentice-Hall, Englewood Cliffs, NJ), 2nd Ed., pp. 556–559.
16. Winer, B. J. (1971) *Statistical Principles in Experimental Design* (McGraw-Hill, New York), 2nd Ed., pp. 196–210, 397–402.
17. Jensen, E. V. (1991) *Curr. Top. Pathol.* **83**, 365–431.
18. Carette, B. & Poulain, P. (1984) *Neurosci. Lett.* **45**, 205–210.
19. Majewska, M. D. & Schwartz, R. D. (1987) *Brain Res.* **404**, 355–360.
20. Majewska, M. D., Mienville, J.-M. & Vicini, S. (1988) *Neurosci. Lett.* **90**, 279–284.
21. Mienville, J.-M. & Vicini, S. (1989) *Brain Res.* **489**, 190–194.
22. Gee, K. W., Joy, D. S. & Belelli, D. (1989) *Brain Res.* **482**, 169–173.
23. Wu, F.-S., Gibbs, T. T. & Farb, D. H. (1991) *Mol. Pharmacol.* **40**, 333–336.
24. Wong, M. & Moss, R. L. (1991) *Brain Res.* **543**, 148–152.
25. Smith, S. S., Waterhouse, B. D. & Woodward, D. J. (1987) *Brain Res.* **422**, 52–62.
26. Hua, S.-Y. & Chen, Y.-Z. (1989) *Endocrinology* **124**, 687–691.
27. Gordon, G. B., Shantz, L. M. & Talalay, P. (1987) *Adv. Enzyme Regul.* **26**, 355–382.
28. Pashko, L. L., Schwartz, A. G., Abou-Gharbia, M. & Swern, D. (1981) *Carcinogenesis* **2**, 717–721.
29. Cherkin, A. & Flood, J. F. (1988) in *Cellular Mechanisms of Conditioning and Behavioral Plasticity*, eds. Woody, C. D., Alkon, D. L. & McGaugh, J. L. (Plenum, New York), pp. 343–354.
30. Roberts, E. (1990) in *The Biological Role of Dehydroepiandrosterone (DHEA)*, eds. Kalimi, M. & Regelson, W. (de Gruyter, Berlin), pp. 13–42.
31. Mattson, M. P., Guthrie, P. B. & Kater, S. B. (1989) *FASEB J.* **3**, 2519–2526.
32. Baldwin, B. A. & Soltysik, S. S. (1966) *Brain Res.* **2**, 71–84.
33. Saffen, D. W., Cole, A. J., Worley, P. F., Christy, B. A., Ryder, K. & Baraban, J. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7795–7799.
34. Morgan, J. I., Cohen, D. R., Hempstead, J. L. & Curran, T. (1987) *Science* **237**, 192–197.
35. Hunt, S. P., Pini, A. & Evan, G. (1987) *Nature (London)* **328**, 632–634.
36. Sagar, S. M., Sharp, F. R. & Curran, T. (1988) *Science* **240**, 1328–1331.
37. Rusak, B., Robertson, H. A., Wisden, W. & Hunt, S. P. (1990) *Science* **248**, 1237–1240.
38. Flood, J. F., Smith, G. E., Bennett, E. L., Alberti, M. H., Orme, A. E. & Jarvik, M. E. (1986) *Pharmacol. Biochem. Behav.* **24**, 631–645.
39. Evans, R. M. (1988) *Science* **240**, 889–895.
40. Beato, M. (1991) *FASEB J.* **5**, 2044–2051.
41. Wahli, W. & Martinez, E. (1991) *FASEB J.* **5**, 2243–2249.
42. Carson-Jurica, M. A., Schrader, W. T. & O'Malley, B. W. (1990) *Endocr. Rev.* **11**, 201–220.
43. Moore, D. D. (1989) *Trends NeuroSci.* **12**, 165–168.
44. Corpechot, C., Synguelakis, M., Talha, S., Axelson, M., Sjovall, J., Vihko, R., Baulieu, E.-E. & Robel, P. (1983) *Brain Res.* **270**, 119–125.
45. Robel, P., Corpechot, C., Synguelakis, M., Groyer, A., Clarke, C., Schlegel, M. L., Brazeau, P. & Baulieu, E. E. (1984) in *Metabolism of Hormonal Steroids in the Neuroendocrine Structures*, eds. Celotti, F., Naftolin, F. & Martini, L. (Raven, New York), pp. 185–194.
46. Robel, P., Bourreau, E., Corpechot, C., Dang, D. C., Halberg, F., Clarke, C., Haug, M., Schlegel, M. L., Synguelakis, M., Vourch, C. & Baulieu, E. E. (1987) *J. Steroid Biochem.* **27**, 649–655.
47. Le Goascogne, C., Robel, P., Gouezou, M., Sananes, N., Baulieu, E.-E. & Waterman, M. (1987) *Science* **237**, 1212–1215.
48. Hu, Z. Y., Bourreau, E., Jung-Testas, I., Robel, P. & Baulieu, E.-E. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8215–8219.
49. Lanthier, A. & Patwardhan, V. V. (1986) *J. Steroid Biochem.* **25**, 445–449.
50. Roberts, E. & Matthysse, S. (1970) *Annu. Rev. Biochem.* **39**, 777–818.
51. Lanthier, A., DiBattista, J. A. & Patwardhan, V. V. (1990) *J. Steroid Biochem.* **35**, 487–494.
52. Demura, T., Driscoll, W. J. & Strott, C. A. (1990) *Endocrinology* **127**, 1114–1120.
53. Oliver, M. F. (1991) *Lancet* **337**, 1529–1531.