indicates that the Long Evans rats have a different (flatter) trend line in addition to being more active overall.

Discussion. We note that all of the albino stocks tested show similar rates and levels of acquisition for the response. That is, the response differences between the F344 and the Sprague-Dawley rats seem only to be statistical, rather than qualitative differences. With the Long Evans strain, the interpretation is not as sure. These animals seem to be showing a slower acquisition pattern while also being less adaptive in terms of immobility. Since Long Evans is the only pigmented stock tested here, and since we have used only albinos in the past, we do not yet know whether the effect we are seeing is due to a pigmented retina (i.e., is it a visual acuity effect?) or whether it is a genotypic (i.e., a strain-typical) activity difference. Thus, we are content to make only two firm points with these data. First, given the similarity between the results from several laboratories in the past[3, 6, 12] with the results of the direct comparisons here, the swimming test can be expected to give a replicable outcome with a variety of rat stocks. Sprague-Dawley rats provide a standard outcome even when they are obtained through different vendor lines. For numerous independent variables then, the test should be effective regardless of locus of origin or the rat stock used.

Secondly, we suggest that this test protocol may provide good basis for continuing behavior genetic work using Norway rats in sleep research. Work with mice *(Mus musculus)* in that area of interest has produced much broadly valuable and elegant research, especially in the domain of psychopharmacology (see, e.g., ref. 19 and its references). In that regard, these data raise interesting questions with respect to albino vs pigmented phenotypes. Early mouse work had suggested that albino animals are more likely to show a passive or hesitant response pattern on a variety of sensory response and learning tasks[20, 21]. The immobility results are compatible with that expectation. Nonetheless, these rats may differ from mice or from other rat strains. As noted, PA is a very active rat strain even though it is albino. In that way it is more like the pigmented Long Evans strain. Thus, in future work, PA might show either the 'albino effect' or the 'strain difference effect' both of which are illustrated by the present data. In resolving that and other biobehavioral questions, we believe that the swimming model will be helpful in elucidating an adaptive, mechanistic, base for a variety of research questions. Traits which are REM sleep sensitive are especially implicated. This, because the REM sleep basis for both response acquisition and emotionality can now be studied simultaneously at the genetic, developmental, neurochemical, and behavioral levels with a reliable rat model.

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2 Hawkins, J., Phillips, N., Moore, J. D., Gilliland, M. A., Dunbar, S., and Hicks, R. A., Physiol. Behav. *25* (1980) 167.
3 Porsolt, R. D., Anton, G., Blavet, N., and Jalfre, M., Eur. J. Pharmac. *47* (1978) 379.
4 Hawkins, J., Hicks, R. A., Phillips, N., and Moore, J. D., Nature *274* (1978) 512.
5 Hodgson, J. A., Fox, R., Hicks, R. A., and Hawkins, J., Sleep Res. *10* (1981) 127.
6 Hodgson, J. A., Physiol. Behav. *32* (1984) 17.
7 Tagney, J., Brain Res. *53* (1973) 353.
8 Gutwein, B. M., and Fishbein, W., Brain Res. Bull. *5* (1980) 9; *5* (1980) 105.
9 Kiyono, S., Seo, M. L., and Shibagaki, M., Physiol Behav. *26* (1981) 391.
10 Mirmiran, M., Scholtens, J., van de Poll, N. E., Uylings, H. B. M., van der Gugten, J., and Boer, G. J., Dev. Brain Res. *7* (1983) 277.
11 Porsolt, R. D., Le Pichon, M., and Jalfre, M., Nature *266* (1977) 730.
12 van Luijtelaar, E. L. J. M., and Coenen, A. M. L., Sleep *8* (1985) 49.
13 Krech, D., Rosenzweig, M. R., and Bennett, E. L., J. comp. Physiol. Psychol. *55* (1962) 801.
14 Fitch, W. M., and Atchley, W. R., Science *228* (1985) 1169.
15 Binik, Y. M., Deikel, S. M., Theriault, G., Shustack, B., and Balthazard, C., Psychophysiology *16* (1979) 381.
16 Richter, C. P., Psychosom. Med. *19* (1957) 191.
17 Griffiths, W. J., Psychol. Rep. *6* (1960) 39.
18 Altman, P. L., and Katz, D. D., Inbred and Genetically Defined Strains of Laboratory Animals. Part 1: Mouse and Rat. Federation of American Societies for Experimental Biology (FASEB), Bethesda, Maryland 1979.
19 Kitahama, K., and Valatx, J.-L., Neuropharmacology *19* (1980) 529.
20 Winston, H. D., Lindzey, G., and Connor, J., J. comp. Physiol. Psychol. *63* (1967) 77.
21 Thiessen, D. D., Owen, K., and Whitsett, M., in: Contributions to Behavior Genetic Analysis, p. 161. Eds G. Lindzey and D. D. Thiessen. Appleton-Century-Crofts, New York 1970.

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Bromocriptine and sulpiride competitively inhibit estrogen binding to its receptor in the adrenal gland

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Summary. Bromocriptine and sulpiride incubated simultaneously with [^{3}H]-estradiol in the cytosol from adrenal glands of adult male rats, yielded curves typical of competitive inhibition as analyzed by Lineweaver-Burk plots. The inhibition constant for both drugs was approximately 10^8 M^{-1}, only 10 times lower than the association constant for estradiol.
Key words. Estrogen receptors; adrenal; bromocriptine; sulpiride.

It is well known that estradiol plays a role in the regulation of the hypothalamic-hypophysial-adrenal axis. It has been reported that ovariectomy causes a decrease of plasma ACTH levels in rats[3]. Homogenates from ovariectomized rats showed a decreased in vitro corticosterone production when compared to homogenates from intact or ovariectomized animals injected with estradiol[4]. Since this effect was evident in homogenates from hypophysectomized-ovariectomized rats receiving both estradiol and ACTH, the estrogen action would appear to occur at least in part directly on the adrenal glands. In agreement with this, estrogen receptors (ER) have been identified[5] and characterized both in the cytosol and in extracts from nuclei of adrenal tissue by our group[6,7] and others[8,9]. Recently, we have studied prolactin regulation of ER in the adrenal gland[10]. This study demonstrated that bromocryptine (BC) and sulpiride (SP) had an effect on the endogenous levels of prolactin. In addition, the effects of these drugs upon adrenal weight and corticosterone secretion could not be ascribed to their action on prolactin levels[10]. Although it is possible that changes with ACTH serum levels occurred, these studies strongly suggest that BR and SP may have a direct effect on the adrenal gland through an interaction with the ER. To assess this hypothesis, we have evaluated

the in vitro effect of BC and SP on cytosolic ER concentration in the adrenal gland of rat.

Materials and methods. Adult male Wistar rats (90 days of age) were fed rat chow and water ad libitum and kept on a schedule of 12 h light:12 h darkness (lights on at 07.00 h) at 23 °C constant temperature. The animals were sacrificed by decapitation and the adrenals were rapidly removed and the fat trimmed off them. The adrenal glands were homogenized in 10 mM Tris-HCl, pH 7.4 containing 0.25 M sucrose, 1.5 mM EDTA, 3 mM $MgCl_2$ and 0.5 mM DTT in the proportion 1:6 (wt/vol), using 10 strokes with an Ultraturrax homogenizer (Janke and Kunkel, IKA Werk, Staufen, FRG). The homogenates were centrifuged at 105,000 × g for 60 min at 4 °C and the cytosol was used to assay for unoccupied ER as previously described[11]. In brief, aliquots of 0.1 ml of cytosol were incubated in duplicate at 4 °C for 16 h with increasing concentrations of [^{3}H] estradiol (0.25–5 nM) in the presence or absence of 500 molar excess of unlabeled 17β-estradiol, for the determination of nonspecific binding, and in the presence or absence of different concentrations of BC or SP. BC was dissolved in absolute ethanol and then diluted up to 30% of ethanol in saline solution (0.9% NaCl). 5 µl of this preparation were added to the appropriate tubes. The same ethanol concentration was added to the control group of tubes. SP was diluted from commercially available vials of Vipral (Roemmers, Buenos Aires, Argentina) in the assay buffer. At the end of the incubation period, an equal volume of dextran-coated charcoal (0.2–2%) in buffer A was added for 10 min at 4 °C. After centrifugation at 800 × g for 15 min, aliquots (100 µl) of supernatant were taken to estimate bound radioactivity. These were pipetted into counting vials containing a mixture of 0.5% PPO and 0.05% POPOP in toluene. Vials were counted in a LS-100 C Beckman Scintillation Counter with 70% efficiency for tritium.

Results were calculated from Lineweaver-Burk plots with linear regression analysis. Inhibition constants were determined by the method of Best-Belpomme and Dessen[12].

Results. To study the in vitro interaction between ER and BC and SP, increasing concentrations of tritiated estradiol were incubated in the presence or absence of fixed concentrations of BC and SP and Lineweaver-Burk analyses of these data were performed.

The analyses obtained for BC are demonstrated in figure 1. All of the competition curves exhibit high correlation coefficients and cross each other at a point near the ordinate axis, all consistent with competitive binding by BC to the ER. As demonstrated in figure 2, SP also results in similar Lineweaver-Burk plots, again suggesting that SP also undergones competitive binding to the ER. Analysis of the inhibition constants (K_i) using the procedure of Best-Belpomme and Dessen[12] revealed K_i is of 0.4×10^8 M^{-1} and 0.5×10^8 M^{-1} for BC and SP, respectively. Both values however are at least 10-fold greater than the association constant of estradiol to its receptor of 0.6×10^9 M^{-1}.

Discussion. BC is a dopaminergic agonist which has been shown to block prolactin release[13], as well as reduce its rate of synthesis[14]. On the other hand, the mechanism of action of SP on prolactin secretion is less clear. It has been reported that although SP has no effect on newly synthesized prolactin in vitro, this drug may overcome the inhibitory effect of dopamine on prolactin secretion[15].

Recently, some direct actions of BC have been reported. Shah and Sheth[16] observed a stimulatory effect of BC on ornithine decarboxylase activity in the ovary and testis using an in vitro assay. At the same time, again using an in vitro assay, results obtained by Bartke et al.[17] and by our group[18], indicate that BC can cause an increase in the testicular androgen production in mouse and rat testis, respectively.

We have previously reported that the injection of BC in immature male rats resulted in a dose-dependent increase in adrenal weight, plasma corticosterone, estrogen and prolactin receptors. However, the opposite effect was observed for SP (only a single dose of SP was used however for this study). The present results may indicate that both drugs act, at least in part, through their competition for estrogen receptors. Whether the importance of this direct effect on the adrenal gland is smaller or larger than the central action on prolactin plasma levels remains to be elucidated.

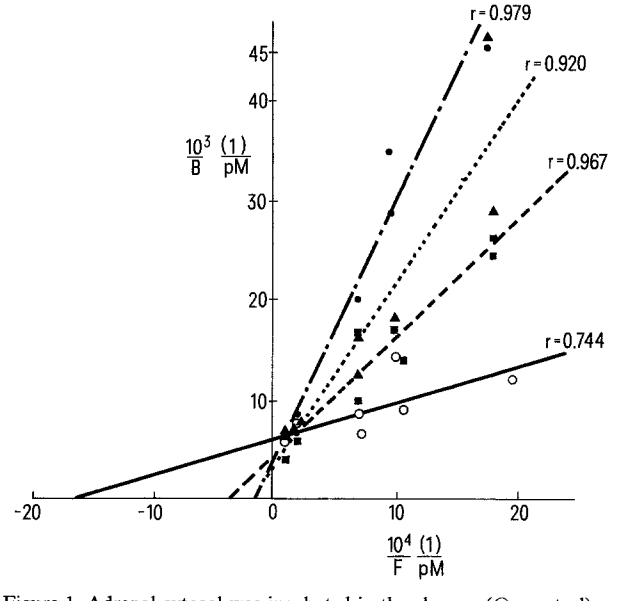

Figure 1. Adrenal cytosol was incubated in the absence (○, control) or presence of BC (■, 10^{-7} M; ▲, 10^{-6} M; ●, 10^{-5} M). [^{3}H]-Estradiol was included simultaneously with BC; for complete details, see Materials and methods. On the ordinate 1/bound [^{3}H]-estradiol and on the abscissa 1/free [^{3}H]-estradiol, for the Lineweaver-Burk plots. The lines were obtained by linear regression, and the experiment was repeated at least twice.

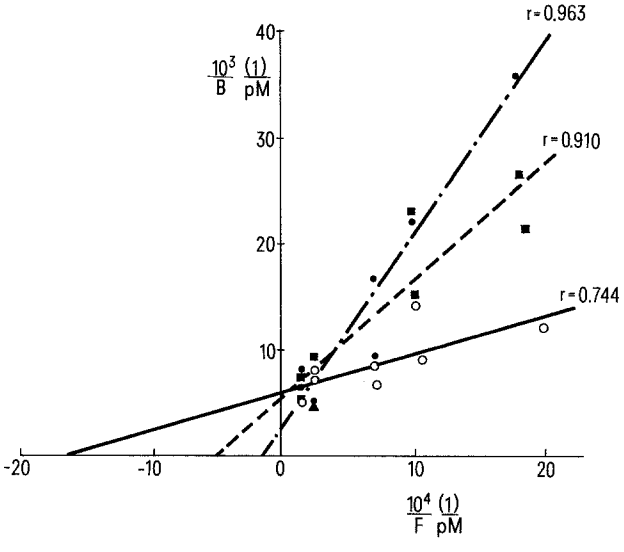

Figure 2. Adrenal cytosol was incubated in the absence (○, control) or presence of SP (■, 10^{-6} M; ●, 10^{-4} M). Details as described in figure 1.

In humans, when BC is administered at a routine dose of 7.5 mg/day (0.15 mg/kg, b.wt), the effective concentration attained is approximately 1×10^{-9} M[19]. If we assume that rats have a similar metabolism of this drug, extrapolation to a dose 10 times greater would yield an effective concentration of 10^{-8} M, which is similar to the inhibition constant estimated by us. Di Carlo et al.[20] have reported a biphasic in vitro effect of BC on the ER from rat uterus. They found that BC was inhibitory at high doses, whereas at low concentrations the binding capacity of the receptor protein increases. This finding is in accordance with the results detected by us in adrenal tissue, using high doses of this drug[10]. Some evidence for a noncompetitive inhibition by BC on the estrogen binding activity in rat uterus has been presented[21]. However, the method of Lineweaver-Burk was developed for measurements of the effect of the concentration of substrates on enzymes, and not for binding studies. Best-Belpomme and Dessen[12] developed calculations which adapt classical inhibition studies to binding assays for receptors or antibodies. Using the Best-Belpomme and Dessen equations, apparent noncompetitive curves found with Lineweaver-Burk analysis, may in fact be of a competitive nature.

We cannot at present explain how drugs with chemical structures different from that of estradiol, can inhibit the binding of this hormone to its own receptor. Nevertheless, a clear inhibition was demonstrated by Lineweaver-Burk analysis. A more reliable approach to the relationship between ER in adrenal gland and dopamine-related drugs could be the measurement of the binding activity in isolated or cultured adrenal cells.

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3 Coyne, M.D., and Kitay, J.I., Endocrinology *85* (1969) 1097.
4 Kitay, J.I., Coyne, M.D., Newsom, W., and Nelson, R., Endocrinology *77* (1965) 902.
5 van Beurden-Lamers, W.M.O., Brinkmann, A.O., Mulder, E., and van der Molen, H.J., Biochem. J. *140* (1974) 495.
6 Calandra, R.S., Naess, O., Purvis, K., Attramadal, A., Djøseland, O., and Hansson, V., J. Ster. Biochem. *9* (1978) 957.
7 Calandra, R.S., Finocchiaro, L.M.E., Lüthy, I.A., and Cheb-Terrab, R., Acta physiol. latinoam. *29* (1979) 333.
8 Müller, R., and Wotiz, H.H., J. biol. Chem. *253* (1978) 740.
9 Cutler, G.B., Barnes, K.M., Sauer, M.A., and Loriaux, D.E., Endocrinology *102* (1978) 252.
10 Lüthy, I.A., Chiauzzi, V.A., Charreau, E.H., and Calandra, R.S., Acta physiol. pharmac. latinoam. *34* (1984) 15.
11 Calandra, R.S., Lüthy, I.A., Finocchiaro, L., and Cheb-Terrab, R., J. Ster. Biochem. *13* (1980) 1331.
12 Best-Belpomme, M., and Dessen, P., Biochimie *55* (1973) 11.
13 Lu, K.-H., Koch, Y., and Meites, J., Endocrinology *89* (1971) 229.
14 Weinstein, D., Schenker, J.G., Gloger, I., Slonin, J.H., De Groot, N., Hochberg, A.A., and Folman, R., FEBS Lett. *126* (1981) 29.
15 MacLeod, R.M., and Robyn, C.J., Endocrinology *72* (1977) 273.
16 Shah, R.G., and Sheth, A.R., Experientia *35* (1979) 1555.
17 Bartke, A., and Lackritz, R.M., Fert. Steril. *35* (1981) 473.
18 Suescun, M.O., Gonzalez, S.I., and Calandra, R.S., manuscript in preparation.
19 Thorner, M.O., Schran, H.F., Evans, W.S., Rogol, A.D., Morris, J.L., and MacLeod, R.J., J. clin. Endocr. Metab. *50* (1980) 1026.
20 Di Carlo, F., Reboani, C., Conti, G., Portaleone, P., Viano, I., and Genazzani, E., in: Pharmacological Modulation of Steroid Action, p. 61. Eds E. Genazzani, F. Di Carlo and W.I.P. Mainwaring. Raven Press, New York 1980.
21 Levy, J., Aflalo, L., and Glick, S.M., Second Innsbruck Winter Conference on Biochemistry in Clinical Medicine (1981), Abstr. 17.

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Proton and potassium fluxes in rat red blood cells incubated with sugar phosphates

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Summary. Fructose-1,6-diphosphate counteracts potassium ejection and proton uptake induced in rat red blood cells by valinomycin and an uncoupler. The effect on potassium ejection is reduced in the presence of ouabain and divalent cations.
Key words. Rat erythrocytes; K^+-flux; H^+-flux; fructose-1,6-diphosphate; potassium ejection.

A previous study[1] showed that fructose-1,6-diphosphate (FDP) induces an uptake of K^+ and an extrusion of H^+ ions detectable, in rat erythrocytes, both in the presence and absence of valinomycin. Further work[2] showed that FDP increases the internal pH and K^+ concentration, and acts as an activator of Ca/Mg-ATPase of human red blood cells. This seems to be a rather specific effect of FDP, which is also bound by the red cell membrane.

An increase of the internal FDP concentration has been observed when FDP is incubated with human red blood cells[3], and FDP is more active than other sugars in protecting the mouse from potassium toxicity[4], since it enhances the uptake of K^+ by the tissues and therefore reduces the hyperkaliemic state[5].

This paper reports the results obtained by measuring K^+ and H^+ fluxes in rat erythrocytes incubated with FDP, fructose + phosphate (F + P), fructose-6-phosphate (F6P) fructose-2,6-diphosphate (F2,6P), an endogenous stimulator of phosphofructokinase[6], and cyclic FDP (FDPc), an intermediate in the chemical synthesis of F2,6P.

Materials and methods. FDP trisodium salt (Biomedica Foscama, Roma), F + P (Boehringer, Mannheim), F6P, FDPc and F2,6P (Sigma, St Louis) were used without further purification. Proton flux was measured with a Beckman glass electrode and potassium flux with a Beckman K^+-sensitive electrode, connected to a Beckman Expandomatic pH-meter and an Omni-Scribe recorder (Houston Instrument). All measurements were carried out at 25 °C, under constant magnetic stirring.

Whole blood was collected from Wistar male rats (280 g), after decapitation, and 5 ml of it was diluted with 30 ml of 125 mM NaCl, 30 mM Tris-HCl pH 8.0 and 10 mM EDTA. After 10 min centrifugation at 2000×g, supernatant and white cells were discarded and the sedimented red cells washed three times with 30 ml of the diluting solution. Hemoglobin (Hb) content was measured according to Beutler[7]. 3–4 mg/ml of Hb were used in each assay and concentration of sugar phosphates ranged between 1 and 4 mM. The experiments were carried out in the presence of 0.3 µg/ml valinomycin and 2 µg/ml carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) (Sigma, St Louis), used together to induce outward K^+ and inward H^+ movements[8] and also in the presence of 66 µM ouabain (Siguama, St Louis), 4 mM $MgCl_2$ and 0.5 mM $CaCl_2$, to inhibit Na/K-ATPase[9] and activate Ca/Mg-ATPase[10]. H^+ translocation was measured in an