8 E. Tria, S. Scapin, C. Cocco and P. Luly, Biochim. biophys. Acta *496*, 77 (1977).
9 H.A. Pershadsingh and J.M. McDonald, Nature *281*, 495 (1979).
10 P. Luly and M. Shinitzky, Biochemistry *18*, 445 (1979).
11 P. Luly, C. Crifò and R. Strom, Experientia *35*, 1300 (1979).
12 D.J. Hanahan and J.E. Eckholm, Meth. Enzymol. *31*, 168 (1974).
13 P. Luly, P. Baldini, S. Incerpi and E. Tria, Biochim. biophys. Acta *555*, 366 (1979).
14 M. Shinitzky and Y. Barenholz, Biochim. biophys. Acta *515*, 367 (1978).
15 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
16 M. Shinitzky and M. Inbar, Biochim. biophys. Acta *433*, 133 (1976).
17 D.E. Atkinson, A. Rev. Biochem. *35*, 85 (1966).
18 R.N. Farias, B. Bloj, R.D. Morero, F. Sineriz and R.D. Trucco, Biochim. biophys. Acta *415*, 231 (1975).
19 L.A. Chen, R.E. Dale, S. Roth and L. Brand, J. biol. Chem. *252*, 2163 (1977).
20 R.A. Cooper, N. Engl. J. Med. *297*, 371 (1977).
21 H.K. Kimelberg, in: Dynamic Aspects of Cell Surface Organisation, p.205. Ed. G. Poste and G.L. Nicolson. North Holland Publishing Co., Amsterdam 1977.
22 H.K. Kimelberg, Biochim. biophys. Acta *413*, 143 (1975).
23 B. Bloj, M.G. Galo, R.D. Morero and R.N. Farias, J. Nutr. *106*, 1827 (1976).
24 T.D. Madden, D. Chapman and P.J. Quinn, Nature *279*, 538 (1979).
25 A. Levitzky and E.J.M. Helmreich, FEBS Lett. *101*, 213 (1979).
26 F. Giraud, M. Claret and K.R. Bruckdorfer, Biochem. Soc. Trans. *8*, 132 (1980).
27 D.E. McMillan, N.G. Utterback and J. La Puma, Diabetes *27*, 895 (1978).
28 Y. Baba, M. Kai, T. Kamada, S. Setoyama and S. Otsuji, Diabetes *28*, 1138 (1979).

In vivo effect of inhibin on FSH uptake by rat testis

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Summary. Simultaneous administration of inhibin and ^{125}I-hFSH significantly decreased the uptake of ^{125}I-hFSH by the immature rat testis. The data suggest that inhibin may modulate FSH action at the gonadal level.

It has now been well established that a gonadal factor, inhibin is involved in the feedback control of FSH secretion. Inhibin acts on the pituitary both in vitro and in vivo as shown by alterations observed in responsiveness to LH-RH[1-4]. However, recent evidence based on in vitro studies suggests that inhibin affects the sensitivity of the testicular tissue to LH as well as FSH[5-8]. Such evidence implies that inhibin may have multiple sites of action. In the present study, we have examined the in vivo effect of inhibin on the uptake of ^{125}I-hFSH by immature rat testis.

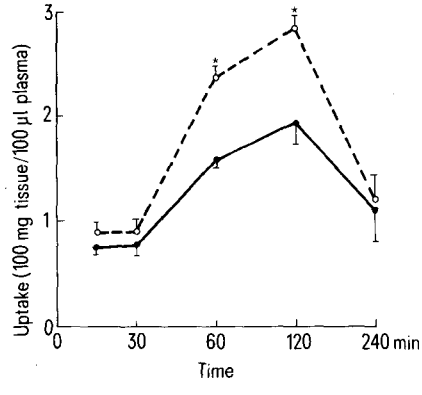
Materials and methods. A homogeneous preparation of inhibin (hSPI) isolated from human seminal plasma, having a mol.wt of 19,000 daltons, was used in the present study[9]. hFSH (NIN-FSH HS-1) kindly supplied by NIAMDD-Bethesda was used as a radioligand. This preparation had a FSH biological activity, as measured in the hCG augmented ovarian weight gain assay[10] of 4990 IU/mg and LH activity as 183 IU/mg as measured by the ovarian ascorbic acid depletion assay[11]. The same preparation was iodinated using the chloramine T method with a sp.act. of 72 μCi/μg. 27-day-old male rats of the Holtzman strain were used. The control animals were injected with single i.v. injection of [^{125}I-hFSH (10 ng) + BSA (50 μg)]/100 μl and experimental animals with [^{125}I-hFSH (10 ng) + hSPI (50 μg)]/100 μl in PBS pH(7.0) respectively via the femoral vein. In both groups animals were bled via heart puncture at different time intervals. Testes were removed, washed with saline and weighed, and the total radioactivity was measured in a γ-ray spectrometer. Radioactivity was expressed as cpm per mg weight or per ml plasma. Uptake was calculated as the ratio of cpm per g weight of tissue/cpm per ml plasma.

Results. Maximum uptake (tissue/plasma) of ^{125}I-hFSH by immature rat testis was 2.8 at 120 min in control rats. Thereafter the uptake of labelled hormone by the testis declined and reached a nadir by 6 h. In the experimental group, simultaneous administration of ^{125}I-hFSH along with inhibin suppressed the uptake of FSH to 1.9 at 120 min (figure). This effect of inhibin on FSH uptake was observed throughout the time interval studied.

In a separate experiment, it was observed that simultaneous injection of 2 μg of unlabelled hFSH via the i.p. route

Specificity of ^{125}I-hFSH uptake by immature rat testis, observation made at 120 min

	Counts per testis ^{125}I-hFSH (10 ng)	^{125}I-hFSH + hFSH (10 ng + 2 μg)
1	4280	751
2	3926	976
3	3776	1121
4	3996	851



Uptake of radioactivity by the testis in control (○---○) and experimental (●——●) animals. The decrease in FSH uptake at 60 min and 120 min was significant, $p < 0.02$ and $p < 0.001$ respectively (Student's t-test). Plotted values represent the mean ± SE for 4 animals.

totally inhibited the uptake of ^{125}I-hFSH indicating specificity of ^{125}I-hFSH uptake by immature rat testis (table).

Discussion. Several reports have appeared on the possible mode of action of inhibin at the level of pituitary[4,6,13,14] and/or hypothalamus[7,14,15]. The results of the present in vivo studies demonstrate that inhibin could significantly affect the FSH uptake by immature rat testicular tissue. The data confirm our earlier report that under in vitro conditions human seminal plasma inhibin suppresses the binding of FSH to testis[19].

It has also been shown that inhibin, both of ovarian and testicular origin, suppresses the binding of FSH to testis and the accumulation of cyclic AMP by testis[7], which suggests that inhibin modulates FSH action at the gonadal level. Irrespective of the mol.wt of inhibin (ram: 1500 daltons, human: 19,000 daltons) a similar action was observed indicating presence of a common biologically active moiety in both preparations.

The present findings suggest that inhibin has multiple sites of action and further knowledge on these lines may lead to our understanding of various reproductive dysfunctions.

1 P. Franchimont, S. Chari and A. Democclin, J. Reprod. Fert. *44*, 335 (1975).
2 P. Franchimont, S. Chari, A.M.C.M. Schellen and A. Demoulin, J. Steroid Biochem. *6*, 1037 (1975).
3 R.V. Davies, S.J. Main, M.G.W.L. Young and B.P. Setchell, J. Endocr. *68*, 26 (1976).
4 H.W.G. Baker, W.J. Bremner, H.G. Burger, D.M. Dekrester, A. Dalmanis, L.W. Eddie, B. Hudson, E.J. Keogh, V.W.K. Lee, G.C. Rennie, Rec. Prog. Horm. Res. *32*, 429 (1976).
5 L.E. Reichert, Jr, and Abou-issa Hussein, Biol. Reprod. *17*, 614 (1977).
6 P. Franchimont, A. Demoulin, J. Verstraclen-Proyard, M.T. Hazee-Hagelstein, J. Walton and G.M.H. Waites, Int. J. Androl. Suppl. *2*, 69 (1978).
7 S. Vijayalakshmi, S.B. Moodbidri, A.H. Bandivdekar and A.R. Sheth, Arch. Androl., in Press (1981).
8 S.B. Moodbidri, L.R. Joshi and A.R. Sheth, Indian J. exp. Biol. *18*, 100 (1980).
9 A.N. Thakur, A.Y. Vaze, B. Dattatreyamurty, N.J. Arbatti and A.R. Sheth, Indian J. exp. Biol. *16*, 854 (1978).
10 S.L. Stellman and F.L. Pholey, Endocrinology *53*, 604 (1953).
11 A.R. Parlow, in: Human pituitary gonadotrophins, p.300. Ed. A. Albert. C. Charles Thomas, Springfield 1966.
12 F.C. Greenwood, M. Hunger and J.C. Glover, Biochem. J. *89*, 144 (1963).
13 S. Chari, S. Duraiswami and P. Franchimont, Horm. Res. *7*, 129 (1976).
14 S.G. Nandini, H. Lipner and N.R. Moudgal, Endocrinology *98*, 1460 (1976).
15 G. Lugaro, M.M. Casellato, G. Mazzola, G. Fachini and G. Carrea, Neuroendocrinology *15*, 62 (1974).
16 A. Arumura, P. Serafini, S. Talbot and A.Y. Schally, Biochem. biophys. Res. Commun. *90*, 687 (1979).
17 C.J. Robert, C. Gloria and K. Karen, Int. J. Fert. *24*, 202 (1979).
18 E.A. Wilson and M.J. Jawad, Fert. Steril. *33*, 91 (1980).
19 S. Vijayalakshmi, P.G. Shah, S.U. Korgaokar, A.Y. Vaze, S.B. Moodbidri and A.R. Sheth, communicated.

Insulin stimulates sodium-potassium activated ATPase from rat hippocampus[1]

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Summary. It is suggested that insulin is capable of activating cerebral NaK-ATPase in a dose-dependent manner.

There is growing interest in a possible physiological relevance of insulin for the central nervous system since it has become evident that nervous tissue contains not only insulin-specific receptors[2-5] but also the hormone itself[6-8]. From findings that insulin does not facilitate the transport of glucose through the blood-brain barrier[9] (BBB), it was concluded that insulin probably fails to act on any kind of brain function. However, the presence of insulin-binding sites in blood vessels[5] and the demonstrated ability of the hormone to stimulate potassium uptake by the brain[10], as well as data about a direct action of insulin on plasma membrane NaK-ATPase in lymphocytes[11], encouraged us to study the effect of the hormone on cerebral NaK-ATPase.

Material and methods. Hippocampi were isolated from rat brain, homogenized in 50 mM Tris HCl buffer (pH 7.4) and centrifuged (20000 × g, 60 min). For comparison, samples were taken from hypothalamus and treated analogously. The determination of ATPase activity was carried out in resuspended sediments from the procedure described above, using the method of Adam-Vizi et al.[12]. Estimation of P_i liberation was performed by the method of Eibl and Lands[13], slightly modified by the authors. Insulin was tested in the following concentrations: 60 μU/ml, 80 μU/ml, 100 μU/ml, 125 μU/ml, 500 μU/ml and, as a really pharmacological reference dose, 20,000 μU/ml. To ensure the effects of insulin on enzymatic activity, an anti-insulin serum with appropriate properties (generated in guinea-pigs) was used as a control substance to paralyze insulin action. The antiserum was utilized diluted 1:100 in distilled water. The protein content of the samples was estimated by the method of Lowry[14]. The statistical treatment of the data was performed by the non-parametric U-test.

Results. The influence of insulin on the activity of sodium-potassium ATPase is summarized in the table. Insulin acts in a dose-dependent manner. At 60 μU/ml the activity was significantly lower than in the controls. 80 μU/ml 'norma-

The effect of various concentrations of insulin on cerebral ATPase

	Insulin (μU/ml)	Percent of basal activity	Significance
Hippocampus	Basal activity: 1.143 ± 0.078 m kat/kg		
	Without	100	-
	60	78	$p < 0.02$
	80	103	no
	100	122	$p < 0.02$
	125	142	$p < 0.01$
	500	172	$p < 0.001$
Hypothalamus	Basal activity: 1.056 ± 0.107 m kat/kg		
	100	129	$p < 0.02$