to $3.4 \times 10^{-3} M$ BANA. Incubation was carried out for 15 min at 37 °C. Enterokinase was determined by its ability to activate trypsinogen. The activation mixtures of 2 ml of $0.02 M$ phosphate buffer (pH 5.8) contained 0.5 mg enterokinase, 0.33–2.0 mg trypsinogen and various amounts of inhibitor. Activation was allowed to proceed for 20 min at 25 °C. The trypsin formed was measured with BANA as substrate. The inhibition constants (K_i) for the amidino compounds were obtained graphically by plotting 1/v against inhibitor concentration at 2 or 3 different substrate concentrations[23]. While these graphs were already indicative of the competitive or noncompetitive nature of the inhibition, confirmation of the type of inhibition was always sought from data arranged in a double reciprocal plot according to LINEWEAVER and BURK[24].

Results and discussion. From the Table it is evident that all new diamidines examined were stronger trypsin inhibitors than either *p*-aminobenzamidine or 4,4′-diamidinodiphenylamine, both of which were included in the study for comparison. The last 5 compounds listed also outranked *p*-amidinophenylpyruvic acid, the K_i value of which with BANA has been reported as $6.0 \times 10^{-6} M$[11]. Amicarbalide and M & B 4596, the only 2 compounds with the amidino groups in the *meta* position, possessed considerable activity against enterokinase but they were not able to match the strength of *p*-amidinophenylpyruvic acid ($K_i = 9.7 \times 10^{-7} M$)[10]. It should be noted that, overall, the inhibitors showed no parallelism between their activity against trypsin on the one hand, and against enterokinase on the other hand. Kinetically, all amidines inhibited BANA hydrolysis by trypsin in a competitive fashion, while against enterokinase only a noncompetitive type of inhibition was encountered. Representative kinetic graphs are demonstrated in the Figure.

The best trypsin inhibitor in the series here, 2,2′-dibromopropamidine, appears to possess about 60% of the activity of the strongest low-molecular weight compound recorded to date, i.e., 4-guanidino-benzoic acid 4′-nitrobenzyl ester[25]. As a reversible inhibitor 2,2′-dibromopropamidine is about equal in potency to the most effective inhibitors produced by BAKER and ERICKSON[15,16] which, like 2,2′-dibromopropamidine, are also phenoxyalkoxy derivatives of benzamidine. Most of their compounds, however, are reversible inhibitors only early during the reaction with trypsin while later on they become covalently bound to the enzyme due to the presence of a fluorosulfonylbenzamide moiety on the second phenyl ring[26].

Inhibition of trypsin and enterokinase by aromatic amidines

Compound	Trypsin K_i (M)	Enterokinase
p-Aminobenzamidine	1.7×10^{-5}	2.0×10^{-5}
4,4′-Diamidinodiphenylamine	6.4×10^{-6}	2.3×10^{-4}
2-Hydroxystilbamidine	6.2×10^{-6}	3.8×10^{-4}
Stilbamidine	6.0×10^{-6}	1.2×10^{-3}
Amicarbalide	4.6×10^{-6}	3.0×10^{-5}
Propamidine	3.3×10^{-6}	1.3×10^{-3}
Pentamidine	2.3×10^{-6}	9.5×10^{-4}
M & B 4596	1.9×10^{-6}	1.1×10^{-5}
2,2′-Dibromopropamidine	7.5×10^{-7}	5.0×10^{-4}

Zusammenfassung. Aromatische Diamidinoverbindungen sind hochwirksame Hemmstoffe des Trypsins. 2,2′-Dibromopropamidin, die stärkste der hier untersuchten Verbindungen, besitzt gegenüber der BANA-Hydrolyse eine Inhibitions-Konstante von $7.6 \times 10^{-7} M$. Amicarbalid und M & B 4596 sind gute Hemmstoffe der Enterokinase, doch erreichen sie die Wirkung der *p*-Amidinophenylbrenztraubensäure nicht.

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Oxidative Activity of Limbic Structures During Sexual Cycle in Rats[1]

It has been demonstrated that the oxygen consumption of hypothalamus undergoes modifications during the sexual cycle in rats[2,3]; lower respiratory rates were observed during diestrus and higher ones during estrus. Apparently the hypothalamic metabolic changes are connected with the mechanisms that control the ovulation and with the sexual hormones that induce the estrus phase[4].

Remembering that the hypothalamus is in relation with the limbic structures and that such structures participate in some way in the ovulatory process[5], it seems to be of interest to determine the oxidative metabolism of the hippocampus and amygdala in female rats during the sexual cycle.

Material and methods. Wistar female rats were used. The animals were fed on the standard diet of the Catedra de Fisiología. Vaginal smears were performed before sacrifice. The animals were decapitated and the amygdala, hippocampus and a sample of cerebral cortex removed. Oxygen uptake was determined by Warburg manometry in vessels of 12–15 ml capacity, containing 3 ml of Krebs-Ringer phosphate buffer, pH 7.4 and 7.7 mM glucose; the central well contained 0.2 ml of saturated

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NaOH solution. The vessels were gassed for 5 min with O_2 100%; 10 min were allowed for the equilibration and the observation period lasted 60 min. All incubations were performed at 37 °C. Results expressed as µl O_2/mg wet tissue/h have been compared with Student's *t*-test accordingly with FISHER and YATES[6].

Results. The oxygen uptake of the amygdala, hippocampus and cerebral cortex from diestrus and estrus rats is summarized in the Table. As can be seen the oxygen uptake of the amygdala is higher during estrus than in diestus phase. On the other hand the oxidative activity of hippocampus is higher during diestrus than in estrus rats. No modifications were found in the respiration of cerebral cortex during sexual cycle.

Discussion. Several observations have demonstrated that the limbic system is one of the brain structures implicated in gonadal function. It was shown that electrical stimulation of the amygdala led to ovulation in the rabbit, the cat and the rat[7–9], and stimulation of the hippocampus induced ovulation in the rabbit[10]. It was also found that bilateral lesions of the hippocampus or amygdala in the adult rat altered the estrus cycle[11,12].

The results of the present paper showed cyclic changes in the oxidative activity of the amygdala and hippocampus. In the amygdala the highest values were observed during estrus and in the hippocampus during diestrus. Such results could be correlated with the observations performed by TERASAWA and TIMIRAS[13] who found that the electrical activity of hippocampus decreases in the morning of the estrus, while the medial part of amygdala decreases its electrical activity during diestrus.

Many functional mechanisms could be implicated from the cyclic changes in the metabolic activity of the limbic structures; nevertheless, taking into account the modifications of sexual hormones and gonadotrophins during the sexual cycle, it is probable that the limbic modifications are in some way connected with such hormonal variations. Further studies are needed before a conclusion can be reached in this respect.

Resumen. Se ha estudiado el consumo de oxígeno de la amigdala y del hipocámpo en ratas hembras durante el ciclo sexual. La corteza cerebral se utilizó como control. Los resultados obtenidos indican que ambas estructuras límbicas sufren cambios cíclicos. La mayor actividad metabólica de la amigdala fué observada durante el estro en cambio el hipocampo tiene su mayor consumo de oxígeno durante el diestro.

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Oxidative activity of limbic structures and cerebral cortex in female rats

	QO_2 (µl O_2/mg wet tissue/h) Amygdala	Hippocampus	Cerebral cortex
Diestrus	1.08 ± 0.11 (15)	1.43 ± 0.10 (26)	1.31 ± 0.12 (13)
Estrus	1.51 ± 0.14 (17)	1.18 ± 0.08 (24)	1.30 ± 0.11 (13)
P value	< 0.02	< 0.05	n.s.

[a] Mean ± standard error. Figures in parenthesis are the number of determinations; n.s., not significant.

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Immunochemical Studies of Ribosomal Proteins Using Passive Hemagglutination Techniques

Immunochemical analysis of ribosomal proteins could be a convenient method for the study of structural similarities between different fractions of ribosomal proteins from the same organism or between ribosomal proteins from different sources, but the use of these techniques has been hindered by the low solubility of ribosomal proteins in acqueous buffers. Unfractionated ribosomal proteins are soluble at fairly high concentrations (> 2 mg/ml) only in urea, in high salt solution, or at extreme values of pH, i.e. under conditions which interfere with the antigen-antibody reaction.

Total ribosomal proteins from *Neurospora crassa*, labelled with ^{14}C dissolved in pyrophosphate buffer at pH 8.5, were assayed in quantitative precipitin reactions, and shown to be all antigenically active, although a considerable fraction of the antigen-antibody complex remained soluble under the conditions of assay[1], therefore preventing any meaningful estimation or comparison.

In this paper we report passive hemagglutination techniques which allow circumvention of some of the difficulties indicated above.

Methods. Ribosomes were isolated from *Neurospora crassa* mycelia and from *Saccharomyces cerevisiae* cells according to a procedure shown to yield ribosomes free from cytoplasmic contaminations[2]. Ribosomes from potato tubers were isolated in the same way, except that $2 \times 10^{-4} M$ natrium diaethyldithiocarbammicum (DIECA) was added to block polyphenoloxidase activities[3] and to avoid the formation of inhibitory quinones[4]. Ribosomes

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