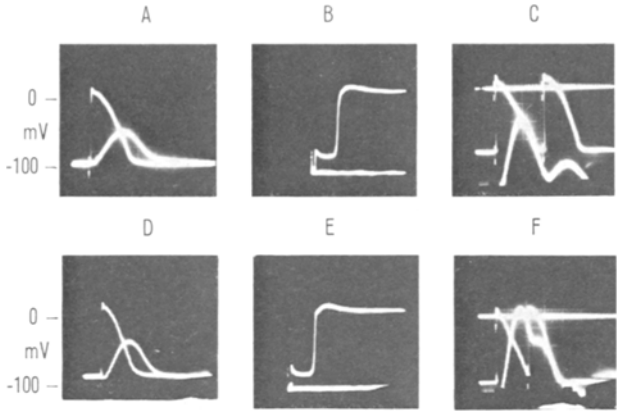

Effect of QTA on the rabbit papillary muscle. Lower row, control records; upper row, 10 min after QTA administration. A, D, action potential and mechanogram; B and E, rapid upstroke of the action potential; C and F, effective refractory period. Time calibration: A, C, D, F: 500 msec; B, E: 10 msec.

quinidine[9,10] and QTA administration is similar. Furthermore, QTA has practically no negative inotropic effect, while quinidine alone causes a significant decrease of the contractility.

Theoretical explanation of the action of QTA is at present impossible, because the essential mechanisms of quinidine action are so far unknown. Theophylline has been found to increase the cellular level of the cyclic-AMP as a result of the inhibition of phophodiesterase activity[6]. It is possible that the action of QTA is due to the interference with the properties of quinidine and cyclic-AMP.

Regardless of the theoretical considerations, it seems important that chemical combinations of some antiarrhythmic drugs with theophylline have better properties than those substances by themselves. Evaluation of the action of some other combinations of the antiarrhythmic drugs with theophylline or other inhibitors of phosphodiesterase may be useful.

Zusammenfassung. Untersuchungen über die Aktion des Chinidin *bis*-(7-theophyllin acetat) auf den isolierten Pappilarmuskel beweisen, dass die Substanz die Verlängerung der Repolarisation, der Refraktärphase, als auch der Kontraktionsdauer bewirkt. Amplitude des Ruhe- und Aktionspotentials, Verlauf der Depolarization und die Kontraktionskraft bleiben praktisch unbeeinflusst.

W. TUGANOWKI, S. KOŚMIDER, M. TENDERA and A. MAŚLANKIEWICZ

Department of Physiology, Silesian Medical School, 19 K. Marksa St., 41-808 Zabrze-Rokitnica (Poland), 15 March 1973.

[9] L. SZEKERES and E. M. VAUGHAN WILLIAMS, J. Physiol., Lond. *160*, 470 (1962).

[10] L. SZEKERES and Gy. J. PAPP, *Experimental cardiac arrhythmias and antiarrhythmic drugs* (Akademiai Kiadó, Budapest 1971).

Interaction of Cocaine and Iproniazid on the H^3-Norepinephrine Uptake Mechanism on Isolated Strip Ventricle of Frog Heart

The catecholamines uptake mechanism by sympathetic nerves of isolated frog ventricle differs in some aspects with the catecholamines uptake mechanism by adrenergic nerves of isolated atrium of guinea-pig[1,2]. In a previous paper[3] we have reported that the potentiation by cocaine of the inotropic response to epinephrine and norepinephrine on isolated frog ventricle was 2,5-fold, while on isolated guinea-pig left atrium it was 30-fold. This fact prompted us to think of a different ability of the blockade by cocaine of catecholamines uptake on the isolated frog ventricle with respect to the isolated atrium of the guinea-pig. In the present work we have studied the blockade by cocaine of the H^3-norepinephrine (H^3-NE) uptake on the isolated ventricle of the frog.

On the other hand, it is established that iproniazid (IPN) inhibits monoamineoxidase and avoids the intraneuronal deamination of catecholamines by these enzymes; so that the norepinephrine and epinephrine uptake and retention by isolated atrium of the guinea-pig is greatly increased[4,5]. In this paper the behavior of the IPN with relation to the H^3-NE uptake and retention by the isolated frog ventricle has also been studied.

Methods. Ventricles of frog (*Rana pipiens*) were prepared and mounted as previously described by FURCHGOTT et al.[6] for isolated atrium of guinea-pig. Ventricles were suspended in an organ bath containing 20 ml of regular Ringer solution of the following composition (expressed in m*M*): NaCl, 103.4; KCl, 1.013; $CaCl_2$, 0.9009; CO_3HNa, 1.851, containing 10^{-5} g/ml of ethylene diaminetetraacetic acid (EDTA). A mixture of 95% O_2 and 5% CO_2 was bubbled through the bathing solution. All preparations were electrically driven at a frequency of 30 beats min. Ventricles were attached to force-displacement transducer (Grass model FT 03), and mechanical activity was recorded by means of a Grass polygraph. Each ventricle was subjected to a resting tension of 1 g. Under their respective conditions, halves were then incubated with 5 ng/ml of D, L-H^3NE for 5 min, and then thoroughly washed. 4 additional washes were given over the subsequent 40 min period, at the end of which the halves were removed for analysis of radioactivity. All preparations were performed at room temperature.

The catecholamine extraction was performed according to the method of ANTON and SAYRE[7] and radioactivity was counted in a Nuclear Chicago Liquid Scintillation Spectrometer model 725. All samples were corrected for quenching with an automatic external reference standard. Under our working conditions, the radioactivity present

[1] R. MARTINEZ-SIERRA and B. LORENZO-VELAZQUEZ, Experientia, *28*, 1063 (1972).

[2] R. MARTINEZ-SIERRA and B. LORENZO-VELAZQUEZ, Experientia *28*, 1456 (1972).

[3] P. SANCHEZ GARCIA, R. MARTINEZ-SIERRA and B. LORENZO-VELAZQUEZ, X Reunión de la sociedad Española de Ciencias Fisiológicas, Valencia 1967, p. 105.

[4] R. F. FURCHGOTT and P. SANCHEZ-GARCIA, J. Pharmac., exp. Ther. *163*, 98 (1968).

[5] R. MARTINEZ-SIERRA, A. VELASCO-MARTIN and P. D. GARCIA DE JALON, Experientia, *29*, 568 (1973).

[6] R. F. FURCHGOTT, S. M. KIRPEKAR, M. RIEKER and A. SCHWAB, J. Pharmac. Exp. Ther. *142*, 39 (1963).

[7] A. H. ANTON and D. F. SAYRE, J. Pharmac. Exp. Ther. *138*, 360 (1962).

in the alumina eluates cannot be ascribed to metabolites of H^3-NE but to H^3-NE itself [8]. H^3-NE is expressed in terms of disintegrations per min per g of tissue (dpm/g). When we refer to H^3-NE uptake, we mean H^3-NE uptake and retention by isolated ventricle of frog heart. Statistical significance of the difference between means was determined by the *t*-test for paired data. The drugs used were: D, L-norepinephrine-7-H^3-hydrochloride, specific activity 16.7 C/mmol (New England Nuclear Corp), iproniazid phosphate (Roche) and cocaine hydrochloride.

Results. In 7 paired experiments, both halves were kept under standard conditions (95% O_2, 5% CO_2 and regular Ringer). After a 30-min period, the experimental half was treated with cocaine (5×10^{-5} g/ml in the organ bath, for 10 min). The other half served as a control. Both halves were then incubated with H^3-NE. Table I shows a statistically significant decrease ($P < 0.05$) between the H^3-NE uptake in preparations treated with cocaine and their controls.

In another series of 9 experiments, performed like the preceding series but treated with iproniazid (5×10^{-4} g/ml in the organ bath, for 20 min) instead of cocaine, the H^3-NE uptake decreased ($P < 0.01$) in preparations treated with IPN with respect to their controls (Table II).

Discussion. Experiments with cocaine. The results obtained show that cocaine greatly blocks the H^3-NE uptake by isolated strip of frog ventricle. This fact does not support our hypothesis that a different ability of blockade by cocaine of the NE uptake of the isolated frog ventricle and of the isolated atrium of guinea-pig was responsible for the differences in the sensitivity to NE observed in both preparations in the presence of cocaine [3].

It is well known that the inotropic response to catecholamines of the frog heart is mediated by β-adrenergic receptors [9]. Consequently, the differences between the sensitivity to NE of the β-adrenergic receptors observed in the atrium of guinea-pig, and that observed in the frog ventricle, are striking. Once having discarded the possibility of a different ability of the blockade by cocaine we could explain these differences if we assume that frog heart has a β-adrenergic receptor less sensitive to NE than the β-receptor from the guinea-pig heart. In addition, the sensitivity of the amphibia heart to NE with respect to isoproterenol, reported by several authors, is not the same; there is a great discrepancy [3, 9, 10]. These facts prompt us to think that in the amphibia there exist factors that under different circumstances could modify the sensitivity to NE of the β-adrenergic receptors. This fact could also be explained by supposing the existence of a extraneuronal *O*-methylation of NE more active in the isolated frog ventricle than in the isolated atrium of the guinea-pig, and assuming that in amphibia this *O*-methylation could be modified under various physiological or experimental circumstances. On the other hand, this hypothesis could explain the variations in concentration of catecholamines reported by several investigators in amphibia [11, 12]. Further investigations are needed to support this hypothesis.

Experiments with iproniazid. The experimental results obtained with isolated ventricle of frog, pretreated with IPN, show that this monoamineoxidase inhibitor behaves as a potent blocker of the uptake of H^3-NE. This finding is unexpected since the pretreatment with IPN greatly increases the H^3-NE uptake and retention by isolated atrium of the guinea-pig [4, 5]. As the experiments were carried out at room temperature (15 °C approximately), in a preliminary publication [11] we suggested that in frog ventricle a certain temperature may be required for the inhibitor effect of IPN to become manifest.

To test this idea, several experiments were conducted at different temperatures (25 °C, 35 °C and, in summer, at room temperature, that is, 20 °C approximately; unpublished results). However, IPN also blocked the H^3-NE uptake in a similar manner to that described in the present paper. This finding provides presumptive evidence that IPN is a blocking agent of the H^3-NE uptake by isolated ventricle of frog. However, by the moment we cannot say whether this blockade is produced by 1. competition with the labelled amine at the level of the transfer site in the neuronal membrane or 2. competition with the H^3-NE at the level of the storage vesicles, accompanied by a decreased or any ability to inhibit monoamineoxidase of adrenergic nerves of the isolated ventricle of frog heart.

Table I. Uptake and retention of H^3-norepinephrine by isolated ventricle of the frog heart pretreated with cocaine

N [a]	Pretreated with cocaine	H^3-NE present during incubation	H^3-NE in tissue 45 min after washout [b] (dpm/g)
7	5×10^{-5} g/ml (10 min)	5 ng/ml (5 min)	73,619 ± 13,583
	Controls	5 ng/ml (5 min)	264,702 ± 62,317

[a] Number of paired experiments. [b] Means ± S.E.M. (see text for further details).

Table II. Uptake and retention of H^3-norepinephrine by isolated ventricle of frog heart pretreated with iproniazid

N [a]	Pretreated with iproniazid	H^3-NE present during incubation	H^3-NE in tissue 45 min after washout [b] (dpm/g)
9	5×10^{-4} g/ml (20 min)	5 ng/ml (5 min)	121,412 ± 30,521
	Controls	5 ng/ml (5 min)	215,422 ± 47,445

[a] Number of paired experiments. [b] Means ± S.E.M. (see text for further details).

Resumen. En ventriculo aislado de *Rana pipiens*, la cocaina bloquea potentemente la incorporación de H^3-norepinefrina. La iproniacida, a su vez, se muestra también como bloqueante de la incorporación y/o retención de H^3-NE en la misma preparación farmacologica. Se sugieren unas hipotesis para explicar los resultados obtenidos.

R. Martinez-Sierra and B. Lorenzo-Velazquez

Department of Pharmacology, Medical School University of Madrid, Ciudad Universitaria, Madrid 3 (Spain), 25 January 1973.

[8] A. R. Wakade and R. F. Furchgott, J. Pharmac. exp. Ther. *163*, 123 (1968).
[9] D. Erlij, R. H. Cetrangolo and R. Valadez, J. Pharmac. exp. Ther. *149*, 65 (1965).
[10] A. M. Lands and S. W. Howard, J. Pharmac. Exp. Ther. *106*, 65 (1962).
[11] R. Martinez-Sierra, Tesis doctoral, Facultad de Medicina, Madrid (1970).
[12] J. A. Izquierdo, I. J. Jofré and C. Acevedo, Libro homenaje al prof. B. Lorenzo-Velazquez (editorial Oteo, Madrid 1971), p. 1041.