

Both enucleation and UV-irradiation have been shown to cause increased water vacuole activity in *Ameba proteus*^{11,12}. The osmolar content of such vacuoles is normal, verifying increased net water transport¹³. Either procedure might be expected to cause net hydrolysis of unstable RNA through physical removal or inhibition of RNA polymerase. Exposure of mammalian cells to amphotericin B causes reduced protein and nucleic acid synthesis in a manner that has been related to cation transport¹⁴. The addition of glucose to the medium will partially protect mammalian cells against the action of ActD¹⁵, perhaps on an osmotic basis. However, it remains to be proven that any of these phenomena are directly related to the osmotic effects of accumulating intra-cellular nucleotides. Hydrolysis of large quantities of nucleic acids almost certainly exposes the cells to significant acid-base changes. *Tetrahymena*, like many cells, contain significant quantities of bound K⁺¹⁶. Release of K⁺ or other cations may also play a role in perturbation of the water controlling mechanisms. The elucidation of these effects remains an intriguing problem for future investigations.

Résumé. L'actinomycine D et les températures d'incubation dépassant l'optimum de croissance causent indépendamment une dégradation de l'ARN instable

chez *Tetrahymena pyriformis* vivant. L'œdème cellulaire se produit simultanément et il est proportionnel au degré de dépolymérisation. Puisque les effets de l'antibiotique et les changements de température sont additifs, ces observations suggèrent que les produits de hydrolyse peuvent aboutir à l'accumulation excessive de l'eau dans la cellule.

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¹¹ R. A. RINALDI, *Expl. Cell Res.* 18, 62 (1959).

¹² R. M. IVERSON, as quoted in L. M. MAYER and R. M. IVERSON, *Experientia* 23, 120 (1967).

¹³ L. M. MAYER and R. M. IVERSON, *Experientia* 23, 120 (1967).

¹⁴ M. LUBIN, *Nature* 213, 451 (1967).

¹⁵ G. R. HONIG and M. R. RABINOVITZ, *Science* 149, 1509 (1965).

¹⁶ P. B. DUNHAM, *Biol. Bull.* 126, 373 (1964).

¹⁷ I thank Miss C. LIPPINCOTT and Mrs. W. ARCHERD for assistance. The Actinomycin D was supplied by Dr. W. B. GALL of Merck, Sharp & Dohme, and the cycloheximide by Dr. G. B. WHITFIELD of Upjohn Co. Supported by USPHS Grant No. GM06461-10. I am currently supported by USPHS Grant No. TO-CA-05117-06/Rad.

Interactions Between Amphetamine and Reserpine in vitro

The in vivo interactions between amphetamine and reserpine have been investigated by a number of workers¹⁻⁵. Contrary to the expected response, administration of reserpine in animals pretreated with amphetamine elicits a marked and prolonged presor response. This effect appears only once. Repetition of reserpine fails to show any response³. The blood pressure increase is blocked and/or prevented by alpha-adrenergic blocking agents; however, it is not modified by bilateral adrenalectomy, decentralization, ganglion blocking agents, antihistaminics and antiserotoninics⁴.

These results seem to indicate that the blood pressure response elicited by reserpine is mainly due to a peripheral adrenergic mechanism. In order to get some more information concerning the mechanism involved in this phenomenon, we tried to reproduce it in vitro using the isolated rat vas deferens preparation, which shows a dense sympathetic innervation⁶ and a high noradrenaline content⁷. The results obtained are reported in this paper.

Material and methods. Male Wistar rats (180-220 g) have been used throughout all experiments. Animals were killed by a blow on the head and both vasa deferentia were removed and suspended in Krebs solution aerated with a mixture of 95% O₂ and 5% CO₂. Bath capacity: 10 ml. Temperature was kept constant at 31 °C. The contractions were registered on a smoked drum by means of a frontal isotonic lever with an 8-fold magnification. The following substances were used: DL-amphetamine sulphate, which in our experimental conditions shows the same activity as the optical isomers, and reserpine as a commercial preparation (Serpasol® Ciba). Solutions were made in isotonic saline 0.9%. Before adding any drug the preparation was allowed to stabilize for 30 min. The sequence of each experiment was as follows: (1) Amphetamine was added to the bath and the response to the concentration used was registered. (2) After washing the preparation, reserpine was added. The time between both drugs differed from one experiment to

another, since relaxation after amphetamine is variable; however, it never exceeded 15 min. Actually, this is not a critical point, since we have observed that even 1 h after addition of amphetamine, reserpine shows the same pattern of activity.

Both drugs were used only once in each preparation and no other substance was employed before the sequence described. The height of the contraction was measured in mm.

Results and discussion. Reserpine, in concentrations up to 3×10^{-4} g/ml, does not elicit any response; however, when added after amphetamine, under our experimental conditions, it produces a sustained contraction of similar character to that determined by amphetamine, i.e. slow, crenated and irregular. The results are shown in the Figure.

Columns on the left side represent the mean height of the contractions obtained with the 3 concentrations of amphetamine used (2.5×10^{-7} , 5×10^{-7} and 1×10^{-6} g/ml, expressed as a base), and the vertical bars are the standard error of the mean. Each column represents the mean of at least 40 experiments.

On the right, the height of the contractions is shown determined by various doses of reserpine following 1 of

¹ F. G. VALDECASAS, J. A. SALVÁ and E. CUENCA, *Arzneimittelforsch.* 140, 581 (1958).

² H. SCHMITT and H. SCHMITT, *Archs int. Pharmacodyn. Théor.* 120, 251 (1959).

³ F. G. VALDECASAS and J. A. SALVÁ, *Archs int. Pharmacodyn. Théor.* 140, 581 (1962).

⁴ F. G. VALDECASAS, J. A. SALVÁ and E. CUENCA, in *Neuro-Psicofarmacology* (Ed. P. B. BRADLEY, P. DENIKER and C. RADOUCO THOMAS; Elsevier, Amsterdam 1959), p. 421.

⁵ A. BONACCORSI, *Europ. J. Pharmac.* 3, 97 (1968).

⁶ K. C. RICHARDSON, *J. Anat.* 96, 427 (1962).

⁷ N. O. SJÖSTRAND, *Acta physiol. scand.* 65, Suppl. 257 (1965).

the 3 doses chosen of amphetamine. Doses of reserpine ranged from 1×10^{-6} to 1×10^{-4} g/ml.

As can be seen, independently of the doses of amphetamine previously used, reserpine response increases in a fashion related to the dose. In the dosage levels of amphetamine used, the maximal response for reserpine corresponds to a concentration of 2.5×10^{-5} g/ml. A higher concentration of reserpine produces a smaller response which may even disappear at a concentration of 1×10^{-4} g/ml. This fact could be explained on the basis that higher concentrations of reserpine produce a blockade of alpha receptors or determine a non-specific effect. The height of the contraction elicited by reserpine has in all cases been smaller than that obtained by amphetamine in the same experiment. Each point of the graph represents the mean \pm the standard error of a minimum of 7 experiments and a maximum of 12.

Further experiments are necessary to elucidate the mechanism involved in the phenomenon described. However, if we take into account the results shown above and some unpublished results, several conclusions may be drawn: (1) The phenomenon appears when amphetamine or amphetamine-like drugs (ephedrine, tranylcipromine) are added before reserpine^{8,9}. (2) Nor-

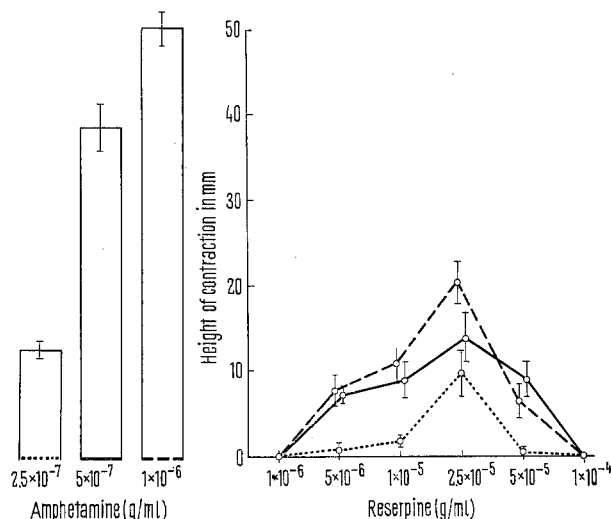
adrenaline and tyramine do not behave like amphetamine⁹. (3) It does not appear when the animals are pretreated with reserpine (1 mg/kg i.p. 24 h before the experiment). (4) The contraction produced by reserpine appears generally once. In some cases a second or even a third dose of reserpine elicit a response, which is always smaller than the previous one. In any case, further doses of reserpine fail to show any response. The weight of the animals and their corresponding vasa deferentia nor-adrenaline content could explain the different number of the responses of the preparations to reserpine. (5) It is blocked by alpha-type adrenolitics (phentolamine).

The abolition of the reserpine response by pretreatment in vivo with the alkaloid and/or pretreatment in vitro with phentolamine, points to an adrenergic mechanism. The possibility that amphetamine modifies the reserpine response through an inhibition of catecholamine uptake or a facilitation of catecholamine release will be investigated¹⁰.

Resumen. Se estudia en el conducto deferente aislado de rata la respuesta a la reserpina después de la estimulación del preparado con anfetamina. En estas condiciones, la reserpina produce una contracción que es proporcional a las concentraciones de anfetamina y reserpina utilizadas, y es máxima para una concentración de reserpina de $2,5 \times 10^{-5}$ g/ml, para ser luego decreciente cualquiera que sea la concentración de anfetamina que se haya empleado previamente. Se discuten los posibles mecanismos que regulan la respuesta a la reserpina después de la anfetamina.

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In vivo interactions between amphetamine and reserpine., after amphetamine (2.5×10^{-7} g/ml); [—], after amphetamine (5×10^{-7} g/ml); - - - - -, after amphetamine (1×10^{-6} g/ml). Each point represents the mean \pm S.E.

⁸ F. G. VALDECASAS, E. CUENCA and L. RODRÍGUEZ, *Actas IX Reunión Nac. Soc. Esp. Ciencias Fisiológicas*, Pamplona 1965, p. 177.

⁹ F. G. VALDECASAS, J. LAPORTE and F. JANÉ, *Actas X Reunión Nac. Soc. Esp. Ciencias Fisiológicas*, Valencia 1967, p. 121.

¹⁰ The authors wish to thank CIBA (Spain) and Laboratorios Miquel S.A. (Spain) for their generous supply of Serpasol® and amphetamine respectively.

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A Neurite-Receptor Complex in the Avian Lung: Electron Microscopical Observations

Electron microscopic observations have demonstrated that the glomus cells of the carotid body¹⁻³ contain many dense-cored granular vesicles and are closely associated with unmyelinated axons. Certain cells in epidermal structures of vertebrates have very similar ultrastructural characteristics, notably Merkel cells in mammalian skin^{4,5} and in the avian hard palate⁶. We have found very similar specialized cells in the epithelium of the intrapulmonary primary bronchus of the domestic fowl (*Gallus domesticus*). Comparable cells have also been described in the human bronchial epithelium⁷.

Specimens of *G. domesticus*, known to be free of certain respiratory diseases, were perfused under barbiturate

anaesthesia with 2.5% glutaraldehyde in cacodylate buffer (pH 7.3). The required tissue was removed, post-fixed in osmium tetroxide and embedded in Maraglas.

¹ J. D. LEVER, P. R. LEWIS and J. D. BOYD, *J. Anat.* 93, 478 (1959).

² F. AL-LAMI and R. G. MURRAY, *J. Ultrastruct. Res.* 24, 465 (1968).

³ T. J. BISCOE and W. E. STEHBENS, *J. Cell Biol.* 30, 563 (1966).

⁴ B. L. MUNGER, *J. Cell Biol.* 26, 79 (1965).

⁵ A. IGGO and A. R. MUIR, *J. Physiol.* 200, 763 (1969).

⁶ A. E. ANDERSEN and P. H. J. NAFSTAD, *Z. Zellforsch. mikrosk. Anat.* 97, 391 (1968).

⁷ K. G. BENSCH, G. B. GORDON and L. R. MILLER, *J. Ultrastruct. Res.* 12, 668 (1965).