pyrophosphatase of the venom is not the essential factor responsible for the inhibition of that mechanism.

Investigations on the characterization of the isolated substance and on the nature and the site of its action are in progress in our laboratory[12].

Résumé. Un inhibiteur de la glycolyse anaérobie est purifié à partir du venin de *Naja naja atra* par un procédé couplant la chromatographie sur SE Sephadex et la filtration sur gel moléculaire. L'inhibition complète de la glycolyse anaérobie est obtenue sur une suspension cellulaire en 60 min pour une concentration inférieure à 20 μg/ml du produit purifié. L'inhibiteur est de nature protéinique et ne présente aucune activité nucléotidasique ni lécithinasique A.

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[12] These experiments were carried out in the laboratory of Biochemistry of the I.I.F.-I.M.C., CERIA, Brussels. We thank the Province de Brabant, the F. W. Breth Foundation, New York, for the financial and moral support given to this work. This work is a part of investigations made by one of us (L. B.) to obtain a doctorate degree.

Positive Correlation of Responsiveness to Catecholamines of the Rat Liver Glycogenolytic Receptor with Other α-Receptor Responses

Current views on the nature of the rat liver glycogenolytic adrenergic receptor are markedly divergent. Some investigators have concluded it is an α-receptor[1-4], while others[5-12] have suggested that it has not yet been adequately characterized. Accordingly, we were prompted to apply the correlation procedure utilized earlier[13,14] which had shown clearly that the adipose tissue lipolytic receptor exhibited the same profile of activity in response to catecholamines as did the cardiac receptor. This had shown lipolysis to be a β-receptor mediated response. In addition, the comparisons yielded clear-cut differentiation of the lipolytic-cardiac receptor from the vasodepressor-bronchodilator one. For simplicity of reference, it was suggested[14] that these different but closely related adrenergic receptors might be termed β-1 and β-2, respectively. More recently, LANDS et al.[15] have provided evidence for an additional β-1 adrenergic receptor, that of rabbit jejunum, while rat diaphragm and uterus were shown to manifest β-2 adrenergic receptor mediated responses.

Data on the delineation of the rat liver glycogenolytic adrenergic receptor are summarized below.

Procedure. For these studies Sprague-Dawley strain male rats, weighing 200–250 g, were maintained on feed and water ad libitum. The test compounds, in 0.01% ascorbic acid stabilized solution, were administered i.p. An hour later blood samples were taken by cardiac puncture and the glucose content, utilized as the index of liver glycogenolysis, determined by the glucose oxidase procedure[16]. In this procedure the glucose is enzymatically oxidized to yield H_2O_2, the oxygen liberated from the latter by catalase being estimated colorimetrically with *o*-dianisidine as the chromogen. The glucostat (Worthington) applied procedure[17] suggested for this series of reactions required modification in our hands to obtain a straight line reference curve. The modifications followed those of FALES et al.[18], utilizing pH 7.0 0.05 *M* phosphate buffer as the reagent diluent and final acidification with 2 drops 4 *N* HCl.

Results. The relative hyperglycemic activities of L-norepinephrine, L-nordefrin, DL-N-methyl-α-methylnorepinephrine and L-N-ethylnorepinephrine compared with a composite epinephrine response (= 100) based on multiple-level dose: response effects are shown graphically in the Figure. Norepinephrine and nordefrin were 1/3 as active as the reference, and N-methyl-α-methylnorepinephrine (as the DL form) and N-ethylnorepinephrine were about 1/4 as active as epinephrine. While epinephrine was active in the same range as that given earlier by ELLIS[19], norepinephrine was considerably more active. ELLIS reported norepinephrine to be 1/15 as hyperglycemic as epinephrine in contrast to the value of 1/3 given here. The reason for the discrepancy is not immediately evident.

The liver receptor was insensitive to L-isoproterenol, to DL-N-tert.butylnorepinephrine and to DL-indolyl-isopropyl-norepinephrine (DL-3,4-dihydroxy-α-([(2-(indol-3-yl)-1-methylethyl)amino]methyl)benzyl alcohol) at

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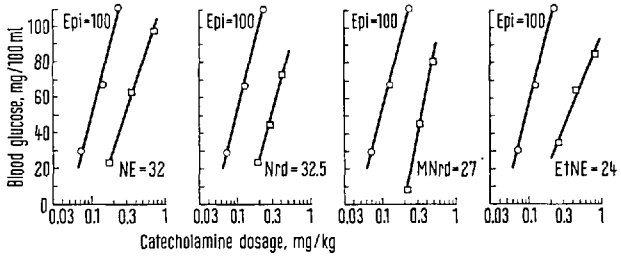
Comparison of activity of 7 catecholamines in rat liver glycogenolysis with that in published α-receptor systems and that in rat lipolysis

Catecholamine[e]	Glycogenolysis	Mouse toxicity[a]	Rabbit ear vasoconstrictor[b]	Rabbit small intestine inhibition[c]	Rat vas deferens contraction[c]	Rat lipolysis[d]
Epinephrine (bitartrate)	100	100	100	300	100	61
L-Norepinephrine (bitartrate)	32	54	50	100	50	85
L-Nordefrin (mucate)	32.5	21				143
DL-N-Methyl-α-methyl-norepinephrine (HCl)	27					45
L-N-Ethylnorepinephrine (HCl)	24	21				235
L-Isoproterenol (bitartrate $2H_2O$)	1	4	1–2[f]			1000
DL-N-*t*-Butylnorepinephrine (methanesulfonate)	1					89

[a] A. M. LANDS and T. G. BROWN[21]. [b] F. P. LUDUENA et al.[22]. [c] J. M. VAN ROSSUM[23]. [d] A. M. LANDS et al.[14]. [e] Salts indicated are those of the rat liver glycogenolysis study. [f] F. P. LUDUENA[29].



Relative rat liver glycogenolytic effects of L-norepinephrine (NE) (n = 31), L-nordefrin (Nrd) (n = 15), DL-N-methyl-α-methylnorepinephrine (MNrd) (n = 14) and L-N-ethylnorepinephrine (EtNE) (n = 16) in comparison with epinephrine (Epi) = 100 (composite responses, n = 59) 1 h after i.p. medication of the rats. Control blood glucose ranged from 105–115 mg/100 ml.

levels of test compound up to 15–30 mg/kg. Earlier[20] indolyl-isopropyl-norepinephrine had been found to be 100 times as hyperglycemic as epinephrine in dogs. On the other hand, the non-catecholamine α-agonists, phenylephrine and naphazoline, were hyperglycemically active. Phenylephrine, in a 3-level dose-response evaluation, was $^1/_{30}$ as active as the reference. Naphazoline, in a single level 1.0 mg/kg dose, was $^1/_{10}$ epinephrine.

Discussion. These findings on the responsiveness of the rat liver glycogenolytic receptor to catecholamines are compared in the Table with the effects of these amines in the commonly accepted α-receptor mediated responses of mouse toxicity, rabbit ear vasoconstriction, rat vas deferens contraction and rabbit small intestine inhibition. In these comparisons isoproterenol and N-tert.butylnorepinephrine are arbitrarily given a value of 1. The rat liver receptor responses are compared also with the representative β-1 receptor response, rat lipolysis. It is seen that the liver receptor response follows that of mouse toxicity closely ($r = 0.942$, $n = 5$, log:log comparison). Similarly, the relative effect of epinephrine, norepinephrine and isoproterenol on the liver receptor is similar to that reported for rabbit ear vasoconstriction, for rat vas deferens and for rabbit small intestine inhibition. On the other hand, the liver receptor manifestly differs from the lipolytic receptor (r near zero, $n = 7$). On the basis of these comparisons it may be concluded that the rat liver receptor can only be viewed as being an α-receptor. The liver glycogenolytic receptor in man has also been proposed as being α[24–26]. Further, ELLIS et al. suggested[9] that the mouse and the rabbit (data of MCCHESNEY et al.[27]) receptors resemble that of the rat, while the cat and dog have liver receptors that are β. Present evidence thus suggests that the nature of the receptor may vary from species to species. And in more general terms, present evidence suggests that receptors that mediate metabolic responses vary from one effect to another. Thus, the rat liver glycogenolytic receptor is α, while the lipolytic receptor is β-1. The suggestion of ROBINSON et al.[28] that α-agonist mediated responses may respond via the adenyl cyclase-cyclic AMP pathway thus finds support in these studies in that the glycogenolytic pathway is considered to be effected via adenyl cyclase-cyclic AMP-phosphorylase[30].

Zusammenfassung. Durch die vollständige Dosis-Wirkungskurve der Katecholamin-i.p.-induzierten Leber-Glykogenolyse an der Ratte konnte deren Einfluss auf die α-Rezeptoren nachgewiesen werden. Da eine Wirkung auf die β-Rezeptoren (Lipolyse) ausschied, konnte es sich nur um einen α-Effekt handeln.

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