Differential inhibitory effects of noradrenaline upon insulin secretion by the isolated perfused pancreas of the rat

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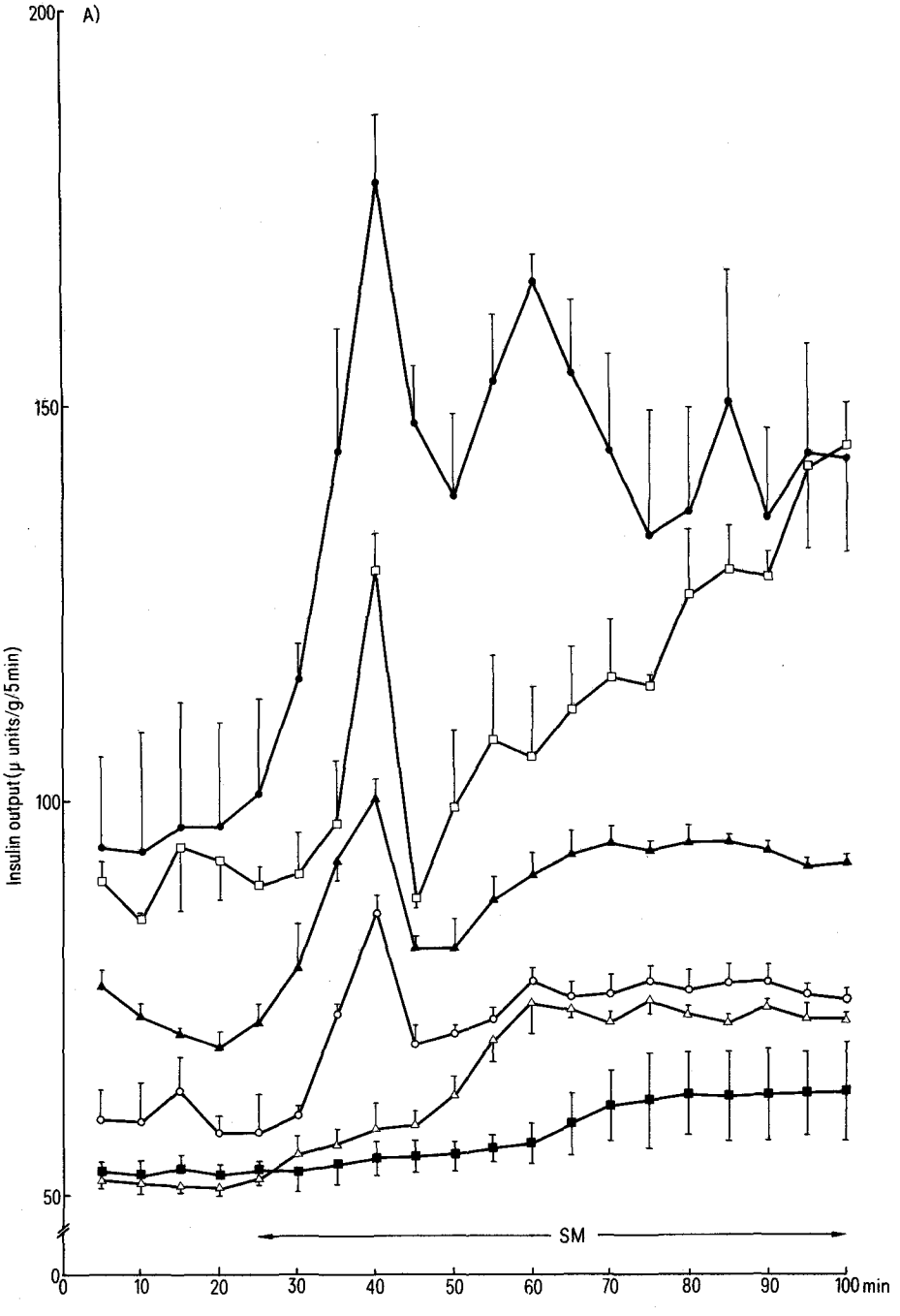
Summary. Responses of basal and glucose-induced secretion phases of the isolated perfused pancreas of the rat to graded doses of noradrenaline (NA) are described. Maximal inhibition of basal secretion was achieved at [NA]=40 ng/100 ml; at [NA]=80 ng/100 ml, the 1st phase of glucose induced secretion was abolished; at [NA]=100 ng/100 ml the 2nd phase was abolished. At [NA] $\leq$ 40 ng/100 ml basal secretion was less sensitive to NA inhibition than glucose induced output.

Noradrenaline (NA) inhibits insulin secretion in vivo[2-4] and in vitro[5]; similarly, splanchnic nerve stimulation inhibits glucose-induced insulin release in calf[6] and dog[7]. NA released at sympathetic terminals appears in blood[8] and plasma concentrations appear to parallel those at such terminals, and are considered to indicate the rate of local NA secretion[9]. Plasma NA in the resting rat is 10-20 ng/100 ml[10]. In both in vivo and in vitro work, NA dose levels used as inhibitors of glucose-induced insulin release have ranged between 30 and 50 ng/100 ml[2,11,12]. No details have



A The effects of increasing NA dose levels upon insulin output by the isolated perfused pancreas. SM indicates period of perfusion with stimulatory medium. ●—● [NA]=0; □—□ [NA]=5; ▲—▲ [NA]=20; ○—○ [NA]=40; △—△ [NA]=80 and ■—■ [NA]=100 ng/100 ml.

been reported of the dose: response relations of NA and insulin secretion. We report here a study of the relationships between dose of NA, and the insulin secretion response of the isolated perfused pancreas.

Materials and methods. 36 male Wistar albino rats (mean b.wt 244.5±5.40 (SEM) g) were randomly divided into 6 groups, each of 6 animals. 1 group was used for each of 6 dose-levels of NA. Following anesthesia with sodium pentobarbitone (6 mg/100 g b.wt, i.p.), the pancreatico-duodenal block was isolated as described by Grodsky et al.[13], and perfused in a 'once-through' system adapted from Sussman et al.[14], with Krebs-Henseleit medium[15] to which was added: a) Dextran T80 4 g/100 ml[13], b) glucose at 5 mmoles/l (basal medium) or 18.87 mmoles/l (stimulatory medium)[16]; c) NA as the acid tartrate, which is stable in solution[17], was included at concentrations of [NA]=0, 5, 20, 40, 80 and 100 ng/100 ml, in both basal and stimulatory media, which were equilibrated with 95% O_2/5% CO_2. Pancreata were perfused with basal medium from 0 to 25 min, and stimulatory medium from 25 to 100 min; perfusate outflows were collected in 5 min batches. Insulin was determined by radioimmunoassay against porcine standards. Insulin outputs were expressed as μU/g wet wt of pancreas/5 min.

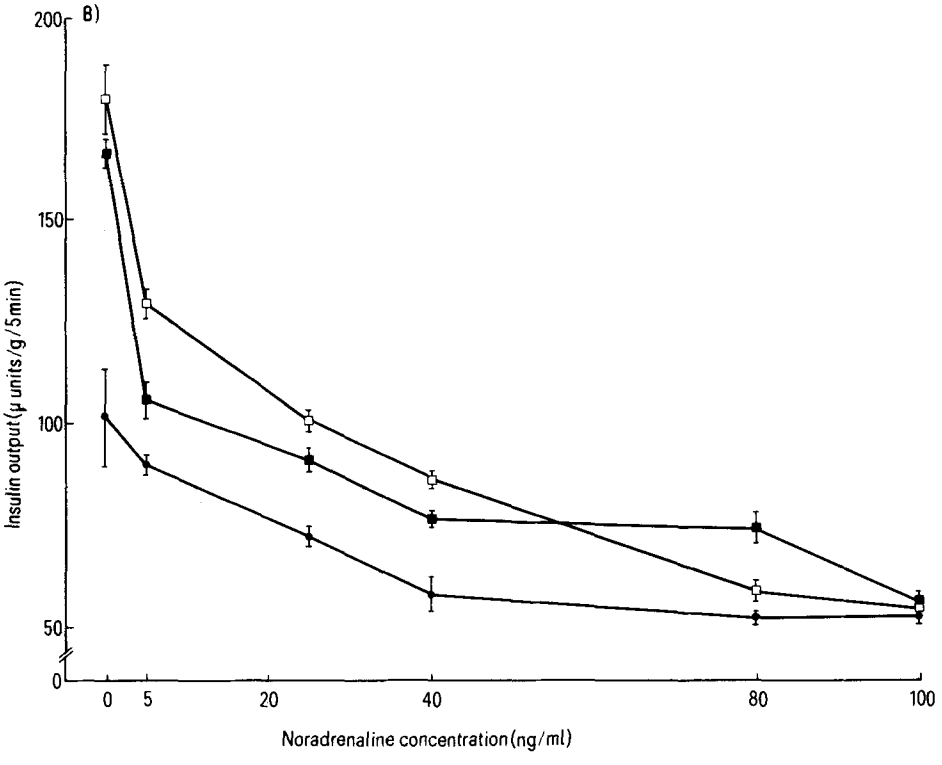
Results and discussion. The results are presented in figure A. The secretion profiles exhibit, at lower [NA], the well documented biphasic pattern following glucose stimulation[18]. Comparisons were made between insulin output levels at 5 min and 20 min (basal secretion), at 40 min, the 1st phase of glucose-induced secretion (phase 1) and at 60 min and 80 min representing commencement, of and steady state 2nd phase of secretion (phase 2).

Analysis of variance of the data indicated highly significant effects of NA upon insulin secretion (F_s [5, 150]=18.6, $p < 0.001$). The time vs [NA] interaction term likewise indicated highly significant effects (F_s [20, 150]=7.9, $p < 0.001$). Figures A indicates differential effects of NA upon different phases of the secretion profile. 1-way analysis of variance of insulin outputs at each of the above-mentioned time points was followed by the use of Duncan's multiple range test (at $p < 0.05$) to identify homogenous subpopulations with respect to [NA] at each time point as summarized in the table.

At 5 min the maximal inhibitory effect was realized at [NA]=40 ng/100 ml. At 20 min, maximal inhibition occurred at [NA]=20 ng/100 ml. At 40 min, [NA] ≤ 40 ng/100 ml had no effect upon phase 1 secretion which was maintained, albeit from lower basal secretion levels. However, [NA] of 80 and 100 ng/100 ml supressed phase 1. With respect to phase 2, it is clear (fig. A and table 1) that there is a continuing depressant effect up to [NA]=20 ng/100 ml. While Duncan's test indicates that at [NA] ≥ 40 ng/100 ml, a progressive reduction in the magnitude of phase 2 secretion occurs, it is not until [NA]=100 ng/100 ml that complete suppression occurs, as confirmed by a within-group t-test against the 45 min output (p always ≥ 0.06). Phase 1 of glucose-induced insulin secretion is therefore

1-Way analysis of variance with respect to insulin output, and identification of homogenous subpopulations by Duncan's multiple range test ($p < 0.05$) at each dose level of NA. Homogenous subpopulations are indicated by under-rules

Time (min)	Analysis of variance F_s [5, 30]	p	Mutliple range test [NA] ng/100 ml
5	12.7	< 0.001	0 5 20 40 80 100
20	13.4	< 0.001	0 5 20 40 80 100
40	124.3	< 0.001	0 5 20 40 80 100
60	147.6	< 0.001	0 5 20 40 80 100
80	23.0	< 0.001	0 5 20 40 80 100



B Basal (●—●); phase 1 (□—□) and phase 2 (■—■) insulin outputs as a function of [NA].
For each data point N=6; vertical bars indicate the SE of the means.

suppressed at lower [NA] than phase 2, implying a differential sensitivity of the 2 phases to NA inhibition.

This is examined further in figure B, which presents insulin outputs as a function of [NA]. Basal secretion is represented by output at 20 min, phase 1 by output at 40 min, and phase 2 by output at 60 min. In all cases there is highly significant correlation between [NA] and ln-transformed output (basal, $r = -0.858$; phase 1, $r = -0.970$; phase 2 $r = -0.916$: p always < 0.001). 2-way analysis of variance of the data in figure B shows a significant output vs time interaction term (F_s [10, 90] = 12.64, $p < 0.001$), indicating significant differences in the slopes of the 3 lines. Significant 2-way interactions are also demonstrated in paired comparisons of the responses: basal vs phase 1, F_s [5,60] = 16.23; basal vs phase 2, F_s [5, 60] = 12.24; phase 1 vs phase 2, F_s [5,60] = 70.35: p always < 0.001. The slopes of the regression lines calculated upon ln-transformed insulin outputs are basal = 1.013, phase 1 = 1.017, phase 2 = 1.019. Sensitivities of the 3 components of the secretion profile to NA inhibition are thus basal < phase 1 < phase 2.

These findings are consistent with earlier in vivo studies upon the effects of NA and alpha-adrenergic blockers on insulin secretion. Endogenous NA has a greater effect upon glucose-induced than basal secretion[12], and in patients with phaeochromocytoma, basal insulin secretion is frequently unaltered, while glucose-induced secretion is substantially reduced[19]. With administration of exogenous alpha-adrenergic agonists, greater inhibition of glucose-induced than basal insulin secretion has been reported[2], supporting the present findings. With alpha-adrenergic blockade in vivo, basal secretion is unaltered, while glucose-induced secretion is markedly enhanced[20,21]. Also, in the human subject, noradrenaline infusion reduces basal insulin secretion by some 50%, while abolishing glucose-induced secretion[22]. The present data therefore provide in vitro evidence for differential sensitivities of basal and glucose-induced phases of insulin secretion to adrenergic inhibition.

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Age-related adrenocortical response to short-term starvation in young rats

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Summary. In a study of the response to short-term starvation in 4-week-old and 7-week-old male rats, it was found that in the younger rats corticosterone levels rose earlier and reached higher levels; they fell after refeeding to a greater extent. Body weight loss followed the same pattern. Younger rats seem to adapt better to fasting and refeeding.

Food deprivation exerts a stimulatory effect on adrenocortical activity[2-6]. Corticosterone levels increase in response to fasting, a) as a result of emotional distress, and b) as a component of the physiological adaptation to lack of food[7]. This hormone plays an important role in the metabolic response to fasting conditions, i.e., in gluconeogenesis[8]. Furthermore, the loss in body weight has been found to correlate with corticosterone levels[3,8]. In this paper, we have compared the effect of short-term starvation, as well as refeeding, on corticosterone levels and body weight in young males of 2 different ages: Post-weaning (4 weeks) and young adult (7 weeks) rats. The effect of chronic underfeeding is also reported.

Male Sherman rats were used, reared in the laboratory under natural light/dark conditions, constant temperature (22 ± 1 °C) and commercial food and tap water 'ad libitum' until the beginning of the experiments, carried out in autumn and winter. The composition of the food, (Extralabo No. 25 biscuits) was as follows: water: 8.2%, fat: 5.6%, minerals: 8.2%, nitrogenous substances: 25%, cellulose: 3.8%, nonnitrogenous extractive: 49.6%. In short-term experiments, 4 weeks (60 ± 10 g) and 7 weeks (141 ± 8 g) old rats were subjected to the following conditions: a) Control-food 'ad libitum' (F), b) 24 h of starvation (S), c) 60 h of starvation, d) 60 h of starvation plus 24 h of food 'ad libitum'. In long-term experiments, 2 groups of 4-week-old rats were nourished for 3 weeks as follows: a): Control = food 'ad libitum', b) 5 g/day of food, given at 09.00 h. The rats were decapitated at the end of the last/fasting period, between 09.00 h and 11.00 h in all cases. Corticosterone assay was carried out in plasma and adrenal homogenates according to the fluorometric method of De Moor[9].