Antagonism of Catecholamine Inhibition of Insulin Secretion by Methysergide[1]

Previous studies with methysergide indicated that it antagonized the actions of serotonin, but it had no effect on the actions of catecholamines, histamine or acetyl choline[2]. Thus, methylergide is thought to be a specific serotonin antagonist and the pharmacological effects of the compound are usually attributed to this action[3]. The specificity of methysergide's antagonistic action was demonstrated utilizing the cardiovascular and gastrointestinal systems, and it is possible that it is not a specific serotonin blocking agent on all systems.

Biogenic monoamines such as serotonin, norepinephrine, and dopamine are present in endocrine cell systems[4], including the islets of Langerhans of the pancreas[5]. Studies from our laboratory have demonstrated that serotonin, norepinephrine, and dopamine inhibit glucose stimulated insulin secretion from the pancreas of a variety of species, and indicate that these compounds may play a physiological role in the regulation of insulin secretion[6-8]. We have demonstrated that alpha adrenergic blocking agents such as phentolamine, block the inhibition of insulin secretion by both indoleamines and catecholamines[9]. The studies herein reported demonstrate that methysergide, previously thought to be a specific antagonist of serotonin, also antagonizes the inhibitory action of dopamine upon insulin release.

Materials and methods. A previously described in vitro pancreas system was used in the present studies[6,8]. The pancreas was removed from male golden hamsters and Swiss-Webster mice anesthetized with ether, and male New Zealand white rabbits anesthetized with pentobarbital. The pancreas was cut into segments weighing 20 to 25 mg and each segment of pancreas then underwent 2 sequential 15 min incubations (initial incubation and treatment incubation). The initial incubation was done in a modified Krebs Ringer Buffer (KRB) containing 0.6 mg/ml glucose. The treatment incubation was modified KRB with 3 mg/ml glucose. When indicated, test substances such as dopamine and/or methysergide were added to the treatment incubation. Aliquots of each incubation media were directly assayed for insulin by a radioimmunoassay technique[6]. In the hamster and rabbit incubations, insulin levels were calculated from a crystalline pork insulin standard curve while the mouse incubations were calculated with a mouse insulin standard. Insulin release was expressed as the difference in insulin release between the treatment and initial incubations and is referred to as Δ insulin release. The Student's t-test was used to compare differences in Δ insulin release between groups of pancreas pieces incubated in various treatment media[10]. When percent inhibition of insulin release was calculated, the following equation was used:

3-Hydroxytyramine hydrochloride (dopamine) was purchased from Calbiochem (Los Angeles, California) and methysergide maleate (Sansert) was a gift from Sandoz Pharmaceuticals (Hanover, N.J.).

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$$\text{Percent inhibition} = \frac{\text{Net insulin release high glucose} - \text{Net insulin release high glucose + inhibitor}}{\text{Net insulin release high glucose}} \times 100$$

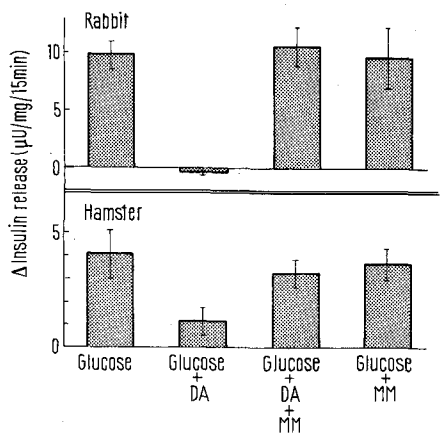
Effect of methysergide on the inhibition of glucose stimulated insulin secretion from mouse pancreas by dopamine

Experiment	Additions to modified KRB with glucose (3 mg/ml)	Concentration (M)	Δ Insulin release[a] (μU/mg/15 min)	Inhibition of insulin release (%)	Significance from modified KRB with glucose (3 mg/ml)
A	None	—	1.7±0.46	—	—
	Methysergide	1×10^{-3}	1.3±0.57	24	N.S.[b]
B	None	—	2.9±0.91	—	—
	Dopamine	1×10^{-4}	0.2±0.58	93	$p < 0.02$
	Dopamine + Methysergide	1×10^{-4} + 1×10^{-4}	0.5±0.42	82	$p < 0.05$
	Methysergide	1×10^{-4}	2.1±0.81	28	N.S.
C	None	—	1.5±0.33	—	—
	Dopamine	2×10^{-5}	0.2±0.20	87	$p < 0.01$
	Dopamine + Methysergide	2×10^{-5} + 1×10^{-4}	0.8±0.29	47	N.S.
D	None	—	4.2±0.97	—	—
	Dopamine	1×10^{-5}	1.0±0.60	76	$p < 0.02$
	Dopamine + Methysergide	1×10^{-5} + 1×10^{-4}	2.7±0.69	36	N.S.

[a] Data are mean ± S.E. of 12 observations in experiment A; 8 observations in experiment B and 11 observations in experiments C and D.
[b] N.S., not significant.

Results. The Figure demonstrates that dopamine inhibits glucose stimulated insulin release from both rabbit and hamster pancreas. This inhibition was blocked by methysergide. In these studies, methysergide alone did not alter glucose stimulated insulin release from either rabbit or hamster pancreas.

The Table shows that methysergide alone did not alter glucose stimulated insulin secretion from mouse pancreas in either $1 \times 10^{-3} M$ (experiment A) or $1 \times 10^{-4} M$ (experiment B) concentration. Experiments B, C and D show that dopamine significantly inhibited insulin secretion when present in concentrations of $1 \times 10^{-4} M$ to $1 \times 10^{-5} M$. With decreasing concentrations of dopamine the constant concentration of methysergide ($1 \times 10^{-4} M$) had a progressively greater ability to antagonize the inhibitory effect of dopamine (experiment B 93% to 82% inhibition, experiment C 87% to 47% inhibition, and experiment D 76% to 36% inhibition). In further studies $1 \times 10^{-5} M$ dopamine was the lowest dopamine concentration that would inhibit insulin secretion from mouse pancreas.



Effect of methysergide (MM) on the inhibition of glucose stimulated insulin secretion from rabbit and hamster pancreas by dopamine (DA). In the upper panel dopamine ($1 \times 10^{-4} M$) inhibited glucose stimulated insulin secretion from rabbit pancreas ($p < 0.01$) and this inhibition was blocked by methysergide. In the lower panel, dopamine ($2 \times 10^{-5} M$) inhibited glucose stimulated insulin secretion from hamster pancreas ($p < 0.05$) and this inhibition was also blocked by methysergide. In these studies the concentration of methysergide used ($1 \times 10^{-4} M$) did not alter glucose stimulated insulin secretion. The glucose concentration was 3 mg/ml. The bars represent the means and the brackets the SE of 6 observations in experiment one and 8 observations in experiment two.

Discussion. The present studies demonstrate that methysergide antagonizes the inhibition of insulin secretion by dopamine. Further studies indicate that methysergide also antagonizes the inhibitory effect of other catecholamines such as L-epinephrine, L-norepinephrine and L-isoproterenol upon in vitro insulin secretion from rabbit pancreas (unpublished observations). Thus, in at least one system (the pancreatic β-cell) methysergide antagonizes the actions of catecholamines as well as serotonin. These data indicate that methysergide cannot be considered as exerting its pharmacologic effects only through serotonin antagonism. The extent to which methysergide antagonizes catecholamine actions in other tissues needs to be reexamined.

Zusammenfassung. Isoliertes Maus-, Hamster- und Kaninchenpankreas wurde in einer physiologischen Pufferlösung mit 3,0 mg/ml Glukose inkubiert. Dopamin ruft eine erhebliche Verringerung der radioimmunologisch reagierenden Insulinsekretion hervor. Die durch Dopamin ausgelöste Hemmung lässt sich teilweise oder vollständig durch den Serotoninantagonisten 1-Methyl-D-lysergsäure butanolamid (Deseril) aufheben.

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The Distribution of Lead in Human Deciduous Teeth

Lead absorbed into the body by various routes is stored in teeth and bone. ALTSHULER et al.[1] have demonstrated substantial increases in the lead content of deciduous teeth of children dying of lead poisoning. Asymptomatic children from areas where lead poisoning is frequent have significantly higher lead levels in shed deciduous teeth than controls from areas in which lead poisoning is unknown[2]. This suggests that the deciduous tooth may provide a means of identifying lead ingestion long after the ingestion has stopped.

It would be of interest to locate the site of lead deposition in teeth as a means of more precisely fixing the time and duration of exposure. This preliminary report describes the use of the electron probe in the study of the distribution of lead in human dental tissues.

Deciduous teeth were cut longitudinally with a diamond saw, shadowed lightly with evaporated carbon, and examined directly in an electron probe microanalyzer (CAMECA). The electron beam voltage was arbitrarily chosen at 30 kV, with specimen currents adjusted to 100–200 nA. The characteristic L-alpha emission line of lead was detected using a quartz crystal focussing spectrometer and side window gas flow proportional counter. Scanning X-ray images were photographed to show localization of lead, phosphorus and calcium.

Twelve specimens from urban children, 2 with known histories of lead poisoning, were examined. Lead was detected in variable amounts in all the teeth. The Figure shows the X-ray images of an area of tooth encompassing enamel, the dentoenamel juction and dentine. In these areas, zones of regular mineralisation (indicated by

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