Effect of Drugs and Catecholamines on Rat Diaphragm Carbohydrate Metabolism

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By the method of paired hemidiaphragms, the mean differences in oxygen and glucose uptake and glycogen content were evaluated for a variety of drugs. Incubation was carried out in a phosphate-saline medium containing 120 mg. per cent glucose. Glycogen was depressed by quinidine sulfate (1.00 mg.), quinine·HCl (1.00 mg.), cinchophen (0.50 mg.), colchicine (0.75 mg.), bishydroxycoumarin (0.25 mg.), dimethyl sulfoxide (1.00 mg.), diphenylhydantoin (0.75 mg.), chlorcyclizine·HCl (0.50 mg.), chlorpromazine·HCl (0.50 mg.), and yohimbine·HCl (1.00 mg.). Glucose utilization was inhibited by the last three agents as well as by tubocurarine chloride (0.50 mg.), picrotoxin (1.00 mg.), atropine sulfate (1.00 mg.), mephenesin carbamate (0.50 mg.), and promethazine·HCl (0.50 mg.). More physiologically significant data resulted with potassium estrone sulfate (10 mcg.) which markedly inhibited glucose utilization and with testosterone (0.52 μm.) and its derivatives, norethandrolone and methandrostenolone, both at 0.33 μm., which depressed glycogen but had little effect on glucose uptake except for testosterone as such. Of the catecholamines, epinephrine at 10 mcg. depressed Q_{O_2}, glycogen, and glucose utilization, the effect persisting at 0.10 mcg. or lower as was also the case with norephinephrine. The latter at 50 mcg. promoted glycogenolysis. Adrenochrome and *dl*-metanephrine·HCl likewise affected glucose uptake at dosages of 10 or 50 mcg., but very high levels of the *O*-methylamine were required for a glycogenolytic response.

ALTHOUGH the isolated rat diaphragm has been used in conjunction with insulin, epinephrine, and several sterols in rather classical muscle metabolic researches, it has been little applied to broader classes of drugs. More recently, findings have been advanced for sulfhydryl compounds (1), thyroid hormones (2), thalidomide (3), and disulfiram (4). This report presents hemidiaphragm oxygen uptake, glucose utilization, and glycogen data for a variety of drugs, therapeutic sterols, and catecholamine derivatives and metabolites screened up to very high levels. The effect of these agents as such or by preincubation on glycogen turnover in the presence of insulin was not ascertained.

EXPERIMENTAL

The drugs were obtained from general commercial sources, mainly Nutritional Biochemicals Corp.[1] The medium of Stadie and Zapp (5) was employed in the incubation of hemidiaphragms, the composition being: 0.04 *M* Na_2HPO_4, 0.005 *M* $MgCl_2 \cdot 5H_2O$, and 0.08 *M* NaCl, pH 6.8–7.0; the glucose content was 120 mg. %.

Male Holtzman rats weighing 135–160 Gm. were starved for 24 hr., sacrificed by swift decapitation, incised, and the hemidiaphragms removed and immediately placed in chilled saline. The tissues were trimmed, blotted between filter paper, weighed, and introduced into the Warburg flasks, one hemidiaphragm being incubated with 1.0 ml. double-strength Stadie and Zapp medium and 1.0 ml. saline as such (control) and its mate, with medium and 1.0 ml. saline–drug solution (treatment). In some instances, as with cinchophen, solution was effected with aqueous NaOH and the mixture then diluted with saline to the desired concentration. A few drugs of low solubility were employed as fine suspensions. With acetylsalicylic acid and the sterols, solutions were prepared in propylene glycol, the desired dosage being delivered in a volume of 0.20 ml.; the respective flasks contained 1.0 ml. of double-strength phosphate–glucose medium, 0.80 ml. of saline, and 0.20 ml. of either glycol or the solution (6). Flasks containing the media but without tissues were also included in each run. The system was gassed with pure oxygen and incubated at 37° for 1 hr., after which time the hemidiaphragms were removed and rinsed 3 times with saline, filter paper blotting being applied between washings. The tissues were digested with alkali and glycogen precipitated and determined by the anthrone reagent (7, 8). The supernatant fluid, after removal of the hemidiaphragm, was deproteinized and analyzed for glucose by the Somogyi method (9, 10). A more detailed description of the pertinent procedures has been advanced earlier (11).

RESULTS

Mean differences in Q_{O_2}, glucose uptake, and glycogen content for hemidiaphragms incubated with drugs, testosterone derivatives, KES, epinephrine, norepinephrine, metanephrine, adrenochrome, and 4-hydroxy-3-methoxymandelic acid together with the requisite Fisher *t* values are presented in Table I. In the calculations involving the bulk data, differences in excess of $\bar{R} \pm 2.5\ R$, where $\bar{R}$ is the average range, were excluded (12). The mean differences were not statistically significant with the following, the values denoting the highest levels screened: acetylsalicylic acid (0.30), acetylcholine (1.00), barbituric acid (0.050), chloral hydrate (0.50), chlorpheniramine maleate (0.50), cyclizine·HCl (0.50), ergotamine tartrate (0.25), sodium heparin (1.00), meperidine (1.00), meprobamate (0.50), meprobamate metabolite (0.50), morphine sulfate (1.00), ouabain (0.50), pentylenetetrazol (1.00), picrotoxin (1.00), pilocarpine·HCl (0.50), procaine·HCl (1.00), reserpine (0.25), strophanthidin (0.25), strychnine sulfate (1.00), and veratrine

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[1] The following originated from the firms as specified: cyclizine·HCl and chlorcyclizine·HCl (Burroughs Wellcome); meprobamate and its metabolite, 2-methyl-2-β-hydroxypropyl-1,3-propanediol dicarbamate (Wallace); dimethyl sulfoxide (DMSO, Baker); phenylbutazone (Butazolidin, Geigy); MER-25 (ethamoxytriphetol, Merrell); norethanedrolone (17α-ethyl-19-nortestosterone, Nilevar Searle); methandrostenolone (1-dehydro-17α-methyltestosterone, Dianabol, Ciba); sodium heparin of 150 U.S.P. units/mg. and epinephrine (Wilson); pentylenetetrazol (Bilhuber, Knoll); mephenesin carbamate (ester of 3-*o*-toloxy-1,2-propanediol, Squibb); potassium estrone sulfate (KES, Penick); chlorpheniramine maleate (Hexagon); and diphenylhydantoin (Dilantin, Parke Davis Co.).

TABLE I.—MEAN DIFFERENCES IN Q_{O_2}, GLUCOSE UPTAKE, AND GLYCOGEN CONTENT OF HEMIDIAPHRAGMS INCUBATED WITH DRUGS AND HORMONES[a,b]

	Compd., mg.	Mean Q_{O_2} Change, μl./mg. Wet Tissue/hr.	t	Mean Glucose Difference,[c] mcg./mg. Wet Tissue/hr.	t	Mean Glycogen Difference, mcg./mg. Wet Tissue/hr.	t
General Agents	Atropine sulfate (1.00)	0.08 ± 0.077 (11)	1.10	1.64 ± 0.387 (11)	4.20[f]	0.13 ± 0.087 (11)	1.40
	Chlorcyclizine·HCl (0.50	0.09 ± 0.039 (12)	2.35[g]	0.31 ± 0.093 (11)	3.34[f]	0.14 ± 0.054 (12)	2.68[g]
	Chlorpromazine·HCl (0.050)	−0.02 ± 0.040 (11)	0.45	0.79 ± 0.281 (12)	2.82[e]	0.07 ± 0.201 (12)	0.36
	Chlorpromazine·HCl (0.50)	−0.02 ± 0.032 (11)	0.64	1.10 ± 0.313 (10)	3.50[f]	0.62 ± 0.201 (11)	3.08[e]
	Cinchophen (0.050)	0.00 ± 0.040 (12)	0.10	...	...	0.00 ± 0.050 (10)	0.07
	Cinchophen (0.50)	0.17 ± 0.070 (11)	2.11	−0.27 ± 0.154 (11)	1.74	0.36 ± 0.161 (12)	5.85[f]
	Colchicine (0.75)	0.04 ± 0.066 (10)	0.59	...	...	0.18 ± 0.068 (10)	2.66[g]
	Bishydroxycoumarin (0.25)	0.00 ± 0.067 (24)	0.02	0.04 ± 0.481 (19)	0.09	0.52 ± 0.141 (23)	3.71[f]
	Digitoxin (0.50)	0.13 ± 0.050 (11)	2.62[g]	0.49 ± 0.655 (11)	0.74	−0.08 ± 0.051 (10)	1.61
	Dimethyl sulfoxide (DMSO; 0.10)	0.03 ± 0.084 (10)	0.39	0.23 ± 0.480 (11)	0.49	0.02 ± 0.122 (10)	0.19
	Dimethyl sulfoxide (1.00)	−0.04 ± 0.041 (32)	0.86	−0.72 ± 0.360 (29)	1.99	0.22 ± 0.091 (29)	2.30[g]
	Diphenylhydantoin (0.75)	−0.01 ± 0.085 (10)	0.13	−0.96 ± 0.580 (11)	1.70	0.73 ± 0.240 (12)	3.02[e]
	Ethyl carbamate (1.00)	0.06 ± 0.056 (18)	1.05	1.15 ± 0.440 (18)	2.60[e]	0.05 ± 0.084 (18)	0.61
	Mephenesin carbamate hemihydrate (0.50)	0.18 ± 0.057 (11)	3.17[f]	1.02 ± 0.148 (12)	6.91[f]	−0.12 ± 0.062 (10)	1.92
	MER-25 (0.10)	0.05 ± 0.030 (11)	1.73	...	...	−0.02 ± 0.020 (9)	0.85
	MER-25 (0.50)	0.14 ± 0.044 (11)	3.12[e]	−0.01 ± 0.316 (11)	0.04	0.17 ± 0.069 (12)	2.48[g]
	Promethazine·HCl (0.50)	−0.05 ± 0.060 (12)	0.86	0.51 ± 0.115 (11)	4.40[f]	−0.05 ± 0.076 (10)	0.68
	Phenylbutazone (0.50)	0.69 ± 0.112 (16)	6.20[f]	0.43 ± 0.524 (9)	0.81	0.30 ± 0.153 (16)	1.94
	Picrotoxin (1.00)	−0.06 ± 0.053 (12)	1.00	0.88 ± 0.301 (12)	2.90[e]	−0.20 ± 0.145 (12)	1.80
	Quinidine sulfate (0.10)	0.13 ± 0.061 (11)	2.16	0.95 ± 0.560 (11)	1.70	−0.02 ± 0.045 (12)	0.40
	Quinidine sulfate (1.00)	−0.23 ± 0.105 (12)	2.20	−0.11 ± 0.435 (12)	0.24	0.87 ± 0.171 (11)	5.10[f]
	Quinine·HCl (0.10)	0.02 ± 0.028 (11)	0.73	...	...	0.31 ± 0.255 (12)	1.20
	Quinine·HCl (1.00)	−0.15 ± 0.096 (11)	1.54	0.52 ± 0.560 (11)	0.93	1.66 ± 0.182 (12)	9.10[f]
	Tubocurarine chloride (0.50)	−0.13 ± 0.080 (12)	1.53	0.92 ± 0.346 (11)	2.65[g]	0.03 ± 0.155 (10)	0.21
	Yohimbine·HCl (1.00)	0.01 ± 0.154 (11)	0.18	1.39 ± 0.370 (12)	3.80[f]	0.44 ± 0.180 (11)	2.40[g]
Sterols	Methandrostenolone (0.10)[d]	0.30 ± 0.123 (15)	2.45[g]	−0.50 ± 0.370 (12)	1.35	0.18 ± 0.066 (15)	2.77[g]
	Norethandrolone (0.10)[d]	0.23 ± 0.051 (21)	4.43[f]	−0.62 ± 0.810 (18)	0.77	0.14 ± 0.048 (20)	3.48[f]
	KES (0.010)	0.05 ± 0.076 (11)	0.63	1.89 ± 0.195 (12)	9.70[f]	0.10 ± 0.211 (12)	0.48
	KES (0.050)	0.03 ± 0.047 (11)	0.60	2.83 ± 0.120 (12)	23.54[f]	0.03 ± 0.061 (12)	0.50
	Testosterone (0.15)[d]	0.20 ± 0.064 (12)	3.13[f]	1.57 ± 0.591 (11)	2.65[g]	0.32 ± 0.096 (11)	3.35[f]
Catecholamines and Derivatives	Epinephrine (1 × 10^{-4})	−0.01 ± 0.036 (14)	0.31	1.98 ± 0.303 (15)	6.55[f]	−0.20 ± 0.152 (12)	1.29
	Epinephrine (0.010)	0.09 ± 0.040 (16)	2.14[g]	1.68 ± 0.412 (17)	4.07[f]	0.16 ± 0.071 (16)	2.22[g]
	Norepinephrine (1 × 10^{-4})	0.10 ± 0.050 (16)	2.06	0.71 ± 0.177 (15)	4.03[f]	0.13 ± 0.161 (11)	0.80
	Norepinephrine (0.050)	0.01 ± 0.033 (22)	0.38	1.14 ± 0.163 (24)	6.96[f]	0.28 ± 0.128 (17)	2.19[g]
	dl-Metanephrine·HCl (0.050)	0.04 ± 0.036 (14)	1.16	1.37 ± 0.248 (15)	5.51[f]	0.13 ± 0.142 (12)	0.88
	dl-Metanephrine·HCl (0.10)	0.03 ± 0.049 (11)	0.69	0.69 ± 0.137 (11)	5.05[f]	0.35 ± 0.243 (11)	1.42
	dl-Metanephrine·HCl (0.50)	0.00 ± 0.048 (17)	0.01	...	...	0.25 ± 0.062 (17)	4.10[f]
	Adrenochrome (0.010)	0.06 ± 0.031 (22)	1.61	1.61 ± 0.236 (23)	6.83[f]	0.17 ± 0.072 (24)	2.40[g]
	Adrenochrome (0.50)	0.08 ± 0.028 (12)	2.95[e]	0.73 ± 0.209 (13)	3.50[f]	0.45 ± 0.105 (13)	4.31[f]
	Adrenochrome semicarbazone (0.015)	0.00 ± 0.070 (12)	0.01	0.97 ± 0.166 (12)	5.86[f]	0.04 ± 0.061 (12)	0.70
	4-Hydroxy-3-methoxy-mandelic acid (1.00)	0.12 ± 0.059 (15)	1.95	0.42 ± 0.358 (15)	1.16	0.18 ± 1.106 (17)	1.71

[a] The means (±S.E.) are deduced from the number of paired hemidiaphragms specified in the parentheses. [b] A negative mean value is indicative of an increase in response in the presence of the drug. [c] Based on the final concentration of the respective media incubated without tissue. [d] In propylene glycol solution. [e] $P < 0.02$. [f] $P < 0.01$. [g] $P < 0.05$.

sulfate (1.00). Acetylsalicylic acid was tested both as a suspension and in propylene glycol mixture.

DISCUSSION

At the concentrations investigated, decreases in diaphragm respiration occurred in the presence of digitoxin, strophanthidin, and phenylbutazone. Of the mean differences, glucose utilization was decreased with promethazine·HCl, picrotoxin, atropine sulfate, and tubocurarine chloride. Chlorpromazine·HCl at 0.50 mg. depressed both muscle glycogen and glucose uptake but when the dosage was lowered to 0.050 mg., only the effect on glucose utilization persisted. Glycogen was depressed on incubation with the higher concentrations of cinchophen (0.50 mg.) as well as with yohimbine·HCl (1.00 mg.), colchicine (0.75 mg.), diphenylhydantoin (1.00 mg.), quinidine sulfate (1.00 mg.), and quinine·HCl (1.00 mg.), the latter two being ineffective at 0.10 mg.; glucose utilization except for yohimbine·HCl was not affected. Glycogen content was decreased but to a small extent with DMSO (1.00 mg.) and MER-25 (0.50 mg.), the last drug also depressing the Q_{O_2}; neither displayed an effect at 0.10 mg.

Both meprobamate and its metabolic product did not significantly alter any of the mean differences. However, mephenesin carbamate (0.50 mg.) depressed glucose utilization as was also observed with ethyl carbamate screened up to 1.00 mg.; Q_{O_2} was decreased in the presence of the former agent. Perhaps at the high levels, carbamate contributes to the diminished glucose uptake and that other structural attributes of the meprobamate molecule might negate the effect. It might be pointed out that the disulfiram metabolite, diethyldithiocarbamate, like the parent compound, was without action on the isolated diaphragm (4).

Regarding the sterols, KES, an interesting water-soluble conjugate of estrone, markedly depressed glucose uptake to the exclusion of any effect on Q_{O_2} and glycogen content at levels down to 10 mcg. (0.027 μm.). Testosterone introduced as a propylene glycol solution elicited inhibitory effects on the three mean differences at 0.15 mg. or 0.52 μm., but the derivatives, norethandrolone and methandrostenolone, each at 0.10 mg. or 0.33 μm., decreased Q_{O_2} and glycogen and produced no effect on glucose uptake. In fact, although not statistically significant, the latter values tended toward increased hexose utilization. In earlier published accounts, rat diaphragm glycogenesis in the presence of insulin and glucose was shown to be reduced by various sterols and the estrogen, stilbestrol (13, 14), and the inhibitory action did not obtain when hemidiaphragms were preincubated with some of these sterols (15). Desoxycorticosterone and an extract of the adrenal cortex were without action on glycogen synthesis in the presence of insulin (16).

The effect of epinephrine on the isolated rat diaphragm has been described by several workers, glucose uptake and glycogen turnover being decreased; the amine antagonizes the action of insulin on glycogenesis (7, 15–18). According to Reisser (16), the substance antagonizing insulin is not epinephrine as such but an oxidized derivative. In the present study, the effects of *dl*-metanephrine·HCl, adrenochrome, and 4-hydroxy-3-methoxymandelic acid (VMA) were ascertained, epinephrine and norepinephrine being included for purposes of comparison under these conditions. Epinephrine at 10 mcg. depressed the glycogen content and glucose utilization was greatly inhibited at dosages below 0.10 mcg. as was also noted with norepinephrine. The latter at 50 mcg. caused a decrease in glycogen. In this regard, both muscle and liver glycogenolysis in the rat is very marked with epinephrine, whereas norepinephrine possesses low activity relative to muscle glycogen but promotes hepatic glycogenolysis (19, 20). Metanephrine·HCl depressed glucose utilization at levels down to 50 mcg., but its glycogenolytic action was noted at higher concentrations (0.50 mg.). The *O*-methylated derivative, like normetanephrine, plays an important role in catecholamine metabolism in the rat and human, and an *O*-methyl transferase system is implicated (21–25). Adrenochrome, the pigment from epinephrine oxidation was similar to the latter amine in causing depressions in both glucose uptake and muscle glycogen at 0.50 mg.; glycogenolysis was definite but of low statistical significance ($p < 0.05$) at 10 mcg. The more stable semicarbazone at a level comparable to the last one (15 mcg.), although markedly inhibiting glucose uptake, was without effect on the glycogen content. However, controls, such as other semicarbazone types or semicarbazide alone, were not instituted, nor were higher concentrations of this derivative screened. The urinary catecholamine metabolite, VMA, was without action on the diaphragm even at a dosage of 1.00 mg.

The data with the above drugs are of great pharmacological interest. Except for the sterols and catecholamines, one is impressed with their relative refractoriness by the metabolic criteria, and where effects could be discerned, these invariably lacked physiological significance in view of the tremendous dosages screened. In contrast to the diaphragm findings, an increase in glucose uptake occurs with rat cerebral cortical slices under the agency of morphine (25); negative data were also obtained earlier with somewhat lower dosages of acetylcholine (16) and with excessive levels of choline (11).

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