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EFFECTS OF DIMETHYLSULFOXIDE ON CYCLIC AMP ACCUMULATION, LIPOLYSIS AND GLUCOSE METABOLISM OF FAT CELLS*

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Abstract—Dimethylsulfoxide (DMSO) decreased insulin-stimulated [1-^{14}C]glucose oxidation and increased lipolysis. DMSO also potentiated the rise in lipolysis due to glucagon, norepinephrine or theophylline. The rise in cyclic AMP levels due to these lipolytic agents was increased by 1.10 M DMSO. This effect was rapid in onset, since increases in cyclic AMP due to DMSO were detected as early as 40 sec after addition of lipolytic agents. The drop in cyclic AMP accumulation seen after addition of propranolol to fat cells incubated with norepinephrine was reduced by DMSO. DMSO inhibited both the soluble and particulate cyclic AMP phosphodiesterase activity present in fat cells. The metabolites of DMSO, dimethylsulfone and dimethylsulfide, did not cause any change in cyclic AMP accumulation due to norepinephrine, indicating that DMSO and not a metabolite was responsible for the observed effects. These data indicate that DMSO at high concentrations inhibited cyclic AMP phosphodiesterase and is a stimulator of cyclic AMP accumulation and lipolysis in fat cells.

Dimethylsulfoxide has been characterized as a dipolar aprotic solvent [1]. It is able to act as a strong hydrogen bond acceptor [2] but possesses no hydrogens suitable to act as hydrogen bond donors. Thus, DMSO is able to compete with water molecules where water is acting as a hydrogen bond acceptor [2]. These factors have been used to explain the ability of DMSO to act as a solvent for many non-polar molecules and its ability to rapidly penetrate biological membranes in high concentration [1–3].

DMSO also has been shown to have a wide variety of direct biological effects [1, 3], including stimulation of melanophore stimulating hormone release [4] and induction of differentiation in various cell types in culture [5, 6].

We decided to test the effects of DMSO on glucose metabolism, lipolysis and cyclic AMP metabolism in isolated fat cells. The two metabolites of DMSO, dimethylsulfone and dimethylsulfide [7], were also tested to determine if these substances might be responsible in part for the observed effects of DMSO.

MATERIALS AND METHODS

Free white fat cells were obtained from 120 to 160 g female Sprague–Dawley rats (Charles River CD strain) fed laboratory chow *ad lib*. White fat cells were isolated by a modification of the procedure of Rodbell [8] from the pooled parametrial adipose tissue of two or more rats. Krebs–Ringer phosphate buffer of the following composition was used in all experiments: NaCl, 128 mM; $CaCl_2$, 1.4 mM; $MgSO_4$, 1.4 mM; KCl, 5.2 mM; and Na_2HPO_4, 10 mM. The buffer was prepared daily and adjusted to pH 7.4 with NaOH after addition of Armour bovine fraction V albumin powder (No. 10101). All incubations were done in duplicate for each experiment at 37° in a shaking incubator in the presence of 0.5 mM glucose.

In the experiments with DMSO, it was necessary to allow the experimental tubes to cool before the addition of hormones or cells because of the large amount of heat generated when DMSO is mixed with aqueous solutions. The pH of the incubation medium was not affected by the addition of DMSO.

Labeled glucose conversion to carbon dioxide and triglyceride content of fat cells were determined as previously described [9]. Samples were also removed at the end of the experiment for glycerol analysis [10].

Total cyclic AMP (cells plus medium) was measured by a modification of the procedure of Gilman [11], using rabbit muscle protein kinase. 0.1 ml of 2 N HCl was added to the tubes prior to placing in a boiling water bath for 1 min. The tubes were allowed to cool and were neutralized with 0.05 ml of 4 N NaOH. Duplicate 20 μl aliquots were removed for determination of cyclic AMP [12]. The cyclic AMP standards were made up in incubation medium treated in the same manner as the unknowns. The free cyclic AMP was separated from the bound cyclic AMP by charcoal adsorption [13].

Membrane-bound adenylate cyclase activity was determined on ghosts prepared by hypotonic lysis of fat cells [14]. The gosts (60 μg protein) were used immediately and incubated for 10 min at 37° in a total volume of 100 μl containing 40 mM Tris buffer (pH 8.0), 5 mM $MgCl_2$, 30 mM KCl, 8 mM creatine phosphate, 1 mg creatine phosphokinase, 1 mM ATP, 1 mM 1-methyl-3-isobutyl xanthine and the test substances. Cyclic AMP was determined after the reaction mixture containing the ghosts was boiled for 3 min and then diluted to a final volume of 1 ml. Twenty-μl aliquots were measured by the protein

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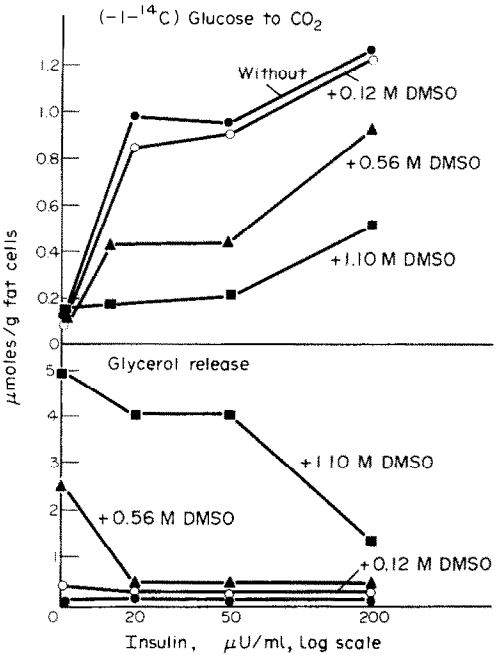

Fig. 1. Effect of dimethylsulfoxide on glucose oxidation and lipolysis. Fat cells (33 mg/tube) were incubated for 1 hr in the presence of 0.5 mM [1-^{14}C]glucose and the concentration of insulin indicated. Dimethylsulfoxide (DMSO) (0.12 M, stars; 0.56 M triangles; or 1.10 M squares) was present from the start of the incubation. The data are the results of two experiments done in duplicate.

kinase binding assay for cyclic AMP. However, the cyclic AMP binding protein was that from the 10,000 *g* supernatant of homogenized bovine adrenal glands. The assay was conducted as described by Brown *et al.* [15] to eliminate interference by ATP.

Soluble cyclic AMP phosphodiesterase was measured using the 48,000 *g* supernatant of homogenized fat cells. The precipitate from the 48,000 *g* centrifugation was washed and resuspended in 2 ml of 40 mM Tris buffer (pH 7.4) and 0.25 M sucrose and was used to assay particulate phosphodiesterase activity. The phosphodiesterase assay was carried out according to the procedure of Thompson and Appleman [16].

Crystalline bovine insulin was a gift of Eli Lilly Co. and contained less than 0.003% glucagon by weight. The other hormones and DMSO were from Sigma Chemical Co.

RESULTS

Figure 1 shows the effect of dimethylsulfoxide on insulin-stimulated glucose oxidation and lipolysis. The concentrations chosen represent 0.8, 4 and 8% of DMSO in the incubation medium. The low dose of DMSO had no significant effect on basal or insulin-stimulated glucose oxidation. This is an important consideration since DMSO has been used as a solvent for many water-insoluble compounds and the data indicate that higher concentrations of DMSO should not be used in experiments with fat cells. The higher concentrations of DMSO did not alter basal glucose oxidation but did inhibit the stimulation by insulin of glucose oxidation. This might have been secondary to activation of lipolysis by DMSO (Fig. 1), since free fatty acids have been shown to inhibit insulin-stimulated glucose oxidation [17]. However, insulin completely blocked the lipolytic effect of 0.56 *η*M DMSO but there was still a decrease in insulin-stimulated glucose oxidation.

Table 1 shows that DMSO is able to potentiate the lipolytic effect of 100 ng/ml of glucagon, 0.15 μM norepinephrine or 50 μM theophylline but does not cause a further increase in lipolysis due to the combination of norepinephrine and theophylline. DMSO was able to potentiate the rise in cyclic AMP elicited by norepinephrine in the presence or absence of theophylline (Fig. 2). There was a significant increase in cyclic AMP due to norepinephrine at 40 sec in the presence of 1.1 M DMSO, while no increase in cyclic AMP could be detected in the absence of 1.1 M DMSO. DMSO also increased cyclic AMP accumulation in the presence of 0.15 μM norepinephrine, 50 μM theophylline or 100 ng/ml of glucagon after a 10-min incubation (data not shown).

To determine the mechanism of this rise in cyclic AMP, the activity of adenylate cyclase in fat cell ghosts was measured in the presence and absence of dimethylsulfoxide. DMSO at 1.3 M inhibited the rise in adenylate cyclase activity due to norepinephrine or glucagon but not that due to fluoride ion (Table 2). There was a slight increase in basal cyclase activity.

The possibility that the rise in cyclic AMP due to DMSO was caused by inhibition of cyclic AMP degradation was tested in the experiments shown in

Table 1. Effect of DMSO on lipolysis*

Additions	Glycerol release (μmoles/g fat cells) Basal	Change due to 1.10 M DMSO
None	0.8 ± 0.6	+1.5 ± 1.1
Norepinephrine (0.15 μM)	9.0 ± 3.6	+18.6 ± 6.3
Theophylline (50 μM)	0.6 ± 0.3	+15.7 ± 0.8
Norepinephrine + theophylline	32.0 ± 3.7	−1.5 ± 1.7
Glucagon (100 ng/ml)	6.3 ± 2.2	+25.5 ± 9.6

* Fat cells (37 mg/tube) were incubated for 1 hr in the presence or absence of 1.10 M DMSO and glucagon, norepinephrine, theophylline or both agents. The data are the mean ± standard error for four experiments done in duplicate.

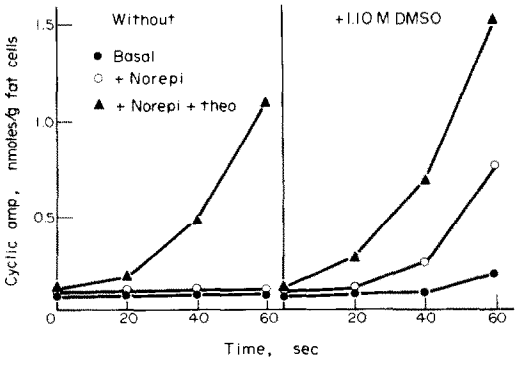

Fig. 2. Effect of DMSO cyclic AMP accumulation at short incubation times. Fat cells (41 mg/tube) were incubated for the times indicated in the presence of norepinephrine (0.15 μM) or norepinephrine (0.15 μM) and theophylline (50 μM). Dimethylsulfoxide (1.1 M) was present where indicated. The data are from a representative experiment done in duplicate.

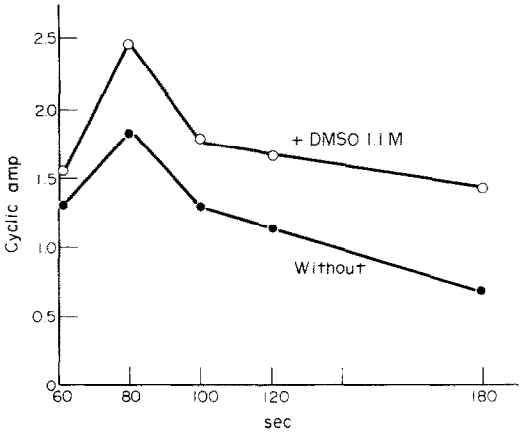

Fig. 3. Inhibition by DMSO of the drop in cyclic AMP seen after the addition of propranolol to fat cells incubated with catecholamines. Fat cells (55 mg/tube) were incubated for 180 sec with 1.5 μM norepinephrine plus 200 μM theophylline. Sixty sec after the start of the incubation, *l*-propranolol (20 μM) was added to all tubes either without or with 1.1 M DMSO. The values are the means of four paired experiments.

Fig. 3. Fat cells were incubated with norepinephrine plus theophylline for 60 sec and then propranolol was added to block adenylate cyclase activation by catecholamine. The results in Fig. 3 indicate that after a 20-sec lag period there was a 50 per cent drop in cyclic AMP due to propranolol alone over the next 100 sec. In other studies (not shown), the values for cyclic AMP progressively increased under identical conditions over the same time period in the absence of propranolol. During the period from 100 to 180 sec the drop in cyclic AMP seen in the presence of propranolol was reduced 50 per cent by 1.1 M DMSO (Fig. 3). A concentration of 0.55 M DMSO gave the same value for cyclic AMP at 180 sec as was seen with 1.1 M DMSO, while 0.27 M DMSO had an inhibitory effect which was only 65 per cent of that seen with 0.55 M DMSO (data not shown). The immediate increase in cyclic AMP seen after DMSO addition might be due to rapid inhibition of cyclic AMP phosphodiesterase or some other effect.

Table 3 shows that DMSO was able to inhibit both soluble and particular cyclic nucleotide phosphodiesterase activity at the two concentrations of cyclic AMP tested. The inhibition due to 1.3 M DMSO was comparable to that of 50 μM theophylline.

The possibility that known metabolites of DMSO (i.e. dimethylsulfone or dimethylsulfide [7]) could be responsible for the effects of DMSO on cyclic AMP accumulation was examined. These compounds paradoxically decreased cyclic AMP accumulation due to norepinephrine. Dimethyllsulfide at a concentration of 100 mM decreased cyclic AMP from 0.38 nmole/g in the presence of norepinephrine (0.15 μM) to 0.05 nmole/g; dimethylsulfone (500 mM) also caused cyclic AMP to decrease to 0.05 nmoles/g when norepinephrine and dimethylsulfone were incubated together. At lower concentrations of these agents (i.e. 100 μM), there was no detectable effect on cyclic AMP accumulation.

Other parameters of lipolysis in fat cells were tested with negative results. DMSO (1.1 M) did not affect the binding of ^{3}H-cyclic AMP to protein kinase isolated from rabbit muscle or the activity of cyclic AMP-dependent protein kinase from fat cells either in the presence or absence of cyclic AMP. There was no effect of DMSO (1.1 M) on the activity of hormone sensitive triglyceride lipase from chicken adipose tissue in the activated (i.e. cyclic AMP present) or in the basal state (Wieser, Malgieri and Shepherd, unpublished observations.

Table 2. Effect of DMSO on adenylate cyclase*

Additions	Basal	% Change due to DMSO	
		0.8 M	1.3 M
None	65	+ 44 (52, 35)	+ 105 (180, 30)
Fluoride, 10 mM	420	+ 17 (33, 0)	+ 32 (64, 0)
Norepinephrine, 100 μM	1185	− 3 (0, −7)	− 36 (−29, −43)
Glucagon, 5 μg/ml	410	+ 6 (12, 0)	− 22 (0, −44)

* Fat cell ghosts (62 μg/tube) were incubated for 10 min in the absence or presence of fluoride or norepinephrine in medium containing 1 mM 1-methyl-3-isobutyl xanthine. Adenylate cyclase activity is expressed as pmoles cyclic AMP accumulated over 10 min. The results are the mean percentage changes due to DMSO and the values in parentheses are the individual values for each experiment.

Table 3. Inhibition of cyclic AMP phosphodiesterase by DMSO*

Source of enzyme	Cyclic AMP (μM)	Phosphodiesterase activity (pmoles/min × mg protein)	% Inhibition by DMSO (1.3 M)
48,000 g Supernatant	0.1	30 ± 6	39 ± 3
48,000 g Supernatant	1.0	181 ± 34	45 ± 2
48,000 g Precipitate	0.1	21 ± 4	35 ± 8
48,000 g Precipitate	1.0	99 ± 24	24 ± 11

* Fat cells were homogenized and then centrifuged at 48,000 g for 30 min. The phosphodiesterase activity during a 10-min incubation of both the precipitate and supernatant was examined at cyclic AMP concentrations of 0.1 μM and 1.0 μM. The effect of DMSO is expressed as per cent inhibition and the data are the mean ± standard error of three experiments.

The stimulation of cyclic AMP accumulation by 1.1 M DMSO was reversible, since in fat cells washed three times after prior incubation with DMSO for 10 min the rise in cyclic AMP accumulation due to norepinephrine and theophylline was actually reduced from a control value of 1.6 to 0.8 nmoles/g. The direct addition of 1.1 M DMSO to these cells increased cyclic AMP by 4.7 nmoles/g in cells previously exposed to DMSO and to 3.5 in control cells. These results indicated that the effects of DMSO are readily reversible and do not result from irreversible inactivation of cyclic AMP phosphodiesterase.

DISCUSSION

The ability of dimethylsulfoxide to inhibit insulin-stimulated glucose oxidation is not the result of the increased lipolysis observed in the absence of insulin with DMSO (Fig. 1). Free fatty acids have been shown to inhibit insulin action on fat cells [17] but DMSO still inhibited insulin-stimulated glucose oxidation under conditions (0.56 M DMSO plus insulin) in which there was no lipolytic action of DMSO.

The lipolytic effect of DMSO and its ability to potentiate lipolysis due to agents such as norepinephrine or glucagon (Table 1) may result from DMSO's potentiation of cyclic AMP accumulation due to these agents (Table 1 and Fig. 2). Cyclic AMP accumulation due to DMSO alone was slight, but there was a potentiation of the rise in cyclic AMP due to norepinephrine or norepinephrine plus theophylline (Fig. 2).

The mechanism by which DMSO increased cyclic AMP accumulation due to catecholamines could result from stimulation of adenylate cyclase activity. However, DMSO actually inhibited the activation of adenylate cyclase by catecholamines. A much more likely possibility is that DMSO inhibits cyclic AMP phosphodiesterase and this effect predominates in intact cells. We suggest that the activation of lipolysis by DMSO is secondary to an elevation of cyclic AMP accumulation resulting from inhibition of cyclic AMP phosphodiesterase.

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