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DMSO as a vehicle for central injections: tests with feeding elicited by norepinephrine injected into the paraventricular nucleus

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Abstract

Dimethyl sulfoxide (DMSO) is becoming increasingly popular as a vehicle in studies employing central injections. The aim of the present study was to determine whether the vehicle required for solubilization of substances for central injection [75% DMSO and 25% artificial CSF (aCSF)] would alter the well-characterized stimulatory response to norepinephrine (NE) injected into the paraventricular nucleus (PVN) on short-term food intake. To evaluate its suitability, we compared the effects of repeated unilateral injections of NE dissolved in two different vehicles (100% aCSF or 75% DMSO, 25% aCSF), in separate groups of animals every 48 h over a 30-day period. NE (40 nmol) stimulated food intake by approximately sevenfold compared to either vehicle alone, and the stimulatory effect was similar whether aCSF or 75% DMSO was used as a vehicle. Furthermore, the NE-induced feeding did not vary in magnitude across a series of 13 tests. These results suggest that 75% DMSO is a suitable vehicle for administering NE (and likely other water-insoluble substances) in small volumes of 0.3 μl into specific brain regions.

Keywords: Appetite; Regulation; Satiety; Brain; Neuropeptide

1. Introduction

The organic solvent dimethyl sulfoxide (DMSO) has been used as a vehicle due to its miscibility with water and ability to increase membrane permeability, thereby facilitating the absorption of compounds that are otherwise insoluble in water. However, numerous studies suggest that DMSO may be toxic or cause nonspecific effects, casting doubt on whether DMSO is indeed a suitable vehicle for the administration of these substances in vivo.

Several studies have previously shown that peripheral injections of DMSO produces toxic and/or adverse effects in a concentration-dependent fashion, but less is known about whether DMSO produces toxic effects following central injections. Peripheral injections of DMSO have been shown to produce tissue (Altland et al., 1966), protein (Friedman, 1968; Hamaguchi 1964), DNA (Walles and Erixon, 1984) and enzyme changes (Altland et al., 1966). Other reported effects include decreases in the formation of cerebral edema, inflammation, platelet association, prostaglandin synthesis, increases in neural tissue blood flow (Willmore and Rubin, 1984) and decreases damage from experimental cerebral infarction and spinal cord injury (Willmore and Rubin, 1984). Decreased growth rate and even mortality have been reported depending on the volume of administration (Weiss and Orzel, 1967, Wyatt and Howarth, 1976). One study, however, reported that DMSO in a concentration-dependent manner protected against cell death caused by intracellular radioisotopes (Bishayee et al., 2000), suggesting that, in some cases, DMSO at certain concentrations may actually benefit cells.

Nonspecific effects have in some cases also been a concern seemingly related to a peripheral route of administration. Studies indicate that DMSO altered levels of luteinizing

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hormone, prolactin and hypothalamic levels of ergocryptine (compared to water; Uphouse et al., 1982), caused hypothermia in rats exposed to cold (Altland et al., 1966) or depressed spontaneous motor activity (Weiss and Orzel, 1967), suggesting that central nervous system activity may be affected following peripheral administration. However, neonatal injections of DMSO did not alter forebrain or hypothalamic weights compared to water alone (Uphouse et al., 1982) nor has peripheral administration of DMSO been found to affect anticholinesterase activity (1 ml/kg ip) or conditioned avoidance responses in rats (5 ml/kg orally) (Uphouse et al., 1982). No differences in the toxicities of various pesticides or drugs (Dixon et al., 1965) were found between DMSO, aqueous suspension (Weiss and Orzel, 1967), corn oil (Weiss and Orzel, 1967) and saline (Dixon et al., 1965), indicating that in some cases DMSO does not produce nonspecific effects. The only study to our knowledge that has examined vehicle effects following central administration reported that DMSO (10%) in 0.5 μl decreased exploratory behavior relative to saline, Tween-80 (2%), propylene glycol (10%) or artificial CSF (aCSF) following injections into the dorsal periaqueductal gray in rats (Matheus et al., 1997). Further studies examining nonspecific effects following central administration will need to be undertaken. These mixed results make it important in cases where DMSO is going to be used as a vehicle, either for central or peripheral injections, to test other appropriate vehicles on the measurement of interest.

Thus, the objective of the present study was to examine whether the vehicle required for solubilization of water-insoluble substances alters the known stimulatory response of norepinephrine (NE) into the paraventricular nucleus (PVN) (Grossman, 1962; Leibowitz, 1978; Varshney et al., 1990; Wellman, 2000) on 1-h food intake. If DMSO is causing toxicity or deleterious effects over time, the food intake response to repeated administration of NE in DMSO would not be expected to be as large as that produced by NE in aCSF and would be expected to decrease as increased damage to the injection site occurs.

2. Material and methods

2.1. Animals

Adult, male Sprague–Dawley rats (Sasco, Omaha, NE) weighing 350–400 g at the time of surgery were provided care according to guidelines established by the Animal Studies Subcommittee of the Omaha VA Medical Center. Rats were housed individually in hanging wire-mesh cages in a temperature-controlled room with a 12/12-h light/dark cycle (lights off 18:00 h).

2.2. Surgical procedures

The procedure for implantation of a guide cannula into a specific brain site has been described previously (Stanley et al., 1989). Each rat was anesthetized with pentobarbital (Nembutal, 50 mg/kg ip) and placed in a stereotaxic apparatus with the incisor bar positioned 3.3 mm below the interaural line. A guide cannula (18 ± 0.05 mm, 26 G, Small Parts, Miami Lakes, FL) was stereotaxically directed to a position 1 mm dorsal to the PVN, which in our rats was 6.9 mm anterior to interaural line, 0.3 lateral to midline and 8.1 mm ventral to skull surface. The cannula was fastened to the surface of the skull with denture acrylic and stainless-steel screws. A 33-G obturator was inserted into the guide cannula to maintain patency. An antibiotic (cefazolin, 250 mg/kg im) was administered at completion of surgery.

2.3. Feeding and experimental protocol

Animals were adapted to a mash diet consisting of Purina rat chow powder (500 g), sucrose (400 g) and evaporated milk (354 ml). Animals were allowed 1–2 weeks of post-operative recovery during which time they were adapted to daily handling and mock injections followed by presentation of food at the beginning of the dark cycle. The experiments were completed over 29 days with injections given during the early part of the light cycle on days following fresh food (provided every 48 h) as follows:

Day	Group 1 ($n = 14$)	Group 2 ($n = 14$)
1	CSF	75% DMSO
3–27	NE in CSF	NE in 75% DMSO
29	CSF	75% DMSO

On experimental days, once food intake had stabilized, food bowls were removed and non-food deprived rats were given injections (bolus injection over a 10 s) directly into the brain through a 33-G injector extending 1 mm beyond the tip of the guide cannula. Food bowls were returned and food intake was measured manually (nearest 0.1 g) for 1-h postinjection.

2.4. Histological verification of PVN cannula placement

The procedure for histological verification of the PVN injection site for each rat has been described previously (Stanley et al., 1989). A subset of animals in both groups ($n = 7/14$) was examined to verify accurate placements within the PVN. There were no gross histological differences between the aCSF- or DMSO-injected groups (Figs. 1 and 2). Although we did not measure the area of the necrotic tissue based on the observations at higher magnification revealed no obvious visual differences between the groups with respect to histological and morphological appearances. The brain tissue appeared normal in both cases, as compared with brain tissue from reference material (PVN of animals that had received multiple injections of NE or CCK-8 in aCSF or threonine in saline) prepared in a

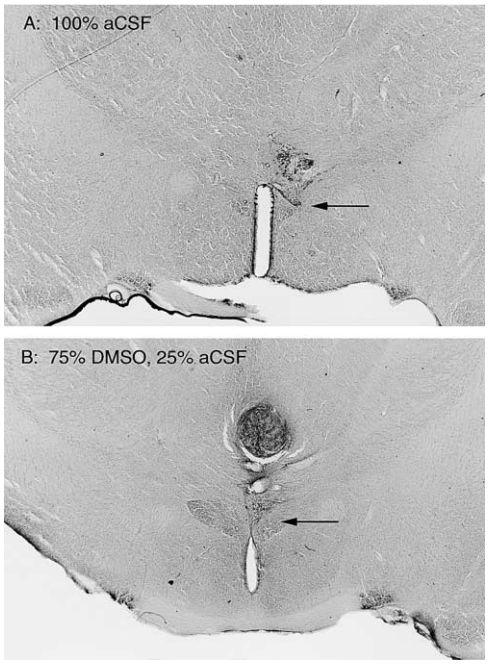

Fig. 1. Photomicrograph of frontal section of the rat brain showing injection site of NE-injected animals with either (A) 100% aCSF or (B) 75% DMSO, 25% aCSF as vehicle. The arrow represents the injection site.

similar way. Nothing was noted visually in either group that suggested damage.

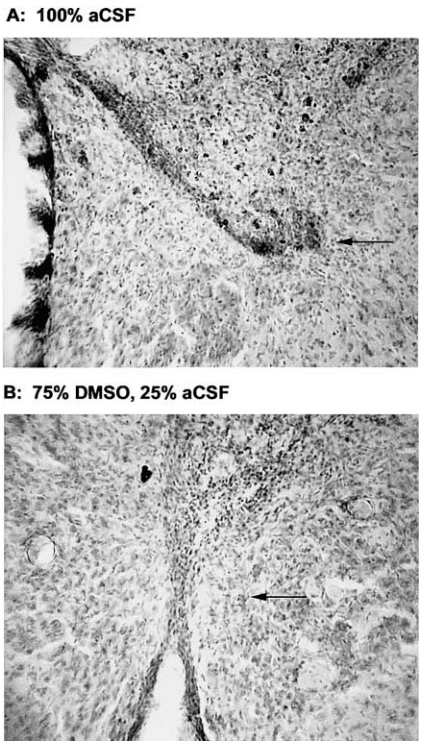

Fig. 2. Photomicrograph of frontal section of the rat brain showing injection site at higher magnification of NE-injected animals with either (A) 100% aCSF or (B) 75% DMSO, 25% aCSF as vehicle. The arrow represents the injection site.

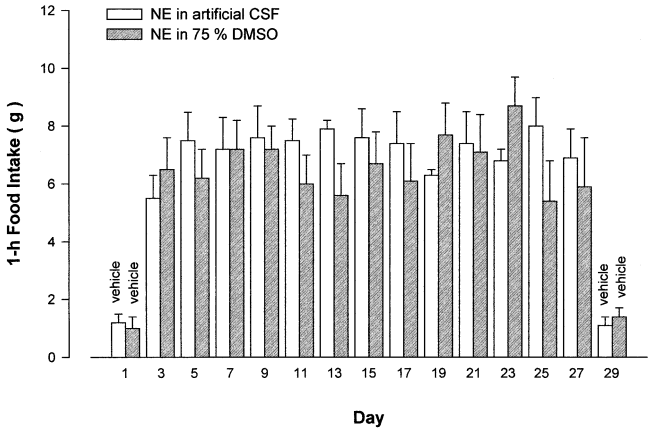

Fig. 3. Effects of PVN injection of NE on food intake. NE (40 nmol in 0.3 μl) was injected in either 75% DMSO or aCSF and food intake was measured 1-h postinjection. Data were analyzed by repeated-measures ANOVA and were highly significant throughout the entire experiment ($P < .00001$ within-subjects day effect compared to control intake).

2.5. Statistical analyses

Values are group means ± S.E. Repeated-measures analysis of variance (ANOVA) was used to evaluate the effects of PVN administration of NE on food intake. Analyses were performed using the statistical program SYSTAT. Differences were considered significant if $P < .05$.

3. Results

3.1. Effects of PVN injection of NE (40 nmol) in 75% DMSO or aCSF on food intake

Fig. 3 shows that injections of NE (40 nmol) into the PVN stimulated food intake by about seven-fold ($P < .00001$). The stimulatory effect of NE was similar whether aCSF or 75% DMSO was used as the vehicle (between subjects, $P > .05$) and NE-induced feeding did not vary across the test period (within subjects, $P > .05$). Likewise, there was no baseline vehicle effect either within or between subjects on days bracketing the NE injections ($P > .05$).

4. Discussion

The aim of this study was to examine whether DMSO can successfully be used as a vehicle for central injections of neuroactive compounds. To accomplish this, we compared the effects of repeated unilateral injections of NE in two different vehicles (100% aCSF or 75% DMSO, 25% aCSF) in separate groups of animals every 48 h over a 30-day period. If DMSO is causing toxicity or deleterious effects over time, two notable outcomes would be expected: (1) The food intake response to repeated administration of NE in

DMSO would not be expected to be as large as that produced by NE in aCSF and it would be expected to decrease over time as increased trauma to the injection area occurs. (2) Gross differences in histology between the two groups should be apparent. These results demonstrate that PVN injection of NE (40 nmol) stimulated food intake similarly whether administered in aCSF or 75% DMSO, and there were no obvious changes to the integrity of the injection site in the DMSO-injected group. Although part of the feeding effect following PVN injections might have been elicited subsequent to NE reflux along the guide cannula into sites dorsal to the PVN (nucleus reuniens; Leibowitz 1978), the NE-elicted feeding effects obtained within the nucleus reuniens or any other sites within a 0.5-mm radius of the PVN (40 nmol) within that study were significantly less than those obtained following PVN injections. Furthermore, 6-OHDA lesions within the PVN, which produced a significant depletion of PVN NE, caused deficits in fasting-induced compensatory feeding and in daily food consumption, suggesting that the PVN is indeed a major area of importance for noradrenergic feeding mechanisms (Shor-Posner et al., 1986). Taken together, these results suggest that DMSO at this concentration does not damage the PVN neurons subserving NE-elicited eating. Thus, it appears to be a suitable vehicle for administering NE (and likely other water-insoluble substances) in small volumes (0.3 μl) into specific brain sites.

These findings are consistent with others that have examined the effects of central injections of various drugs administered in DMSO on food intake or intestinal motility. None of these studies, however, compared the effects of the drug in DMSO to that of the drug in another vehicle, allowing for the possibility that the drug in DMSO might behave differently than if it were dissolved in another solution. However, many if not all the findings concerning the actions of the CCK antagonists, NMDA NR2B/NR2A receptor subunit and glycine site antagonists, and stimulators of cAMP are consistent with respect to these agents not acting in a nonspecific manner. Schick et al. reported that bilateral lateral hypothalamic injection of the CCK-B receptor antagonist, L-365,260 (0.5 μl), dissolved in 100% DMSO, stimulates food intake, an action consistent with the ability of CCK to inhibit food intake following unilateral LH administration (Blevins et al., 2000; Schick et al., 1990). In addition, bilateral ventromedial hypothalamic injection (0.25 μl) of the CCK-A receptor antagonist, devazepide, also dissolved in pure DMSO, inhibited the duration of intestinal motility (Liberge et al., 1990, 1991). This same group also found that intracerebroventricular injection of devazepide, but not L-365,260, dissolved in pure DMSO, decreased the fed state as well as the CCK-induced state of colonic hypermotility (Gue and Bueno, 1991). Compounds such as the protein kinase A inhibitor H-89 and 1,9-dideoxyforskolin, an inactive analog of forskolin, both dissolved in DMSO, produced differential effects on forskolin-activated feeding and H-89 was ineffective at inhibiting nocturnal feeding (Gillard et al., 1998b). Compounds that stimulate cAMP, MPB forskolin (dissolved in aCSF) and IBMX (dissolved in DMSO) potently stimulated food intake when given together at doses that were ineffective when given alone (Gillard et al., 1997), also a finding specific to the perifornical nucleus (PFH; Gillard et al., 1998a). Additionally, the NMDA receptor glycine site antagonist, 7-CK, dissolved in DMSO, elicited opposing actions on feeding in satiated vs. fasted rats and on NMDA- vs. KA-stimulated feeding (Stanley et al., 1997). Ifendprodil tartrate (NMDA NR2B and/or NMDA NR2A receptor subunit antagonist) decreased NMDA- but not KA-elicited eating (Khan et al., 1999). Third ventricular injections of pure DMSO did not affect food intake relative to preinjection baseline intake (personal communication, Dr. David Cummings). DMSO injections into the nucleus accumbens did not alter fixed interval food reinforcement behaviors relative to sham microinjections or post-surgery baseline values (Cory-Slechta et al., 1997). Ritter et al. found that an 80% DMSO, 20% NaCl solution (used to dissolve devazepide and L-365,260), did not produce any signs of discomfort or intolerance following either lateral or fourth ventricular injections, whereas animals injected with this high DMSO concentration intraperitoneally did appear to be in discomfort (Brenner and Ritter, 1998). Taken together, these studies suggest that various compounds within DMSO have selective actions and that DMSO is unlikely to be acting nonspecifically to affect food intake. Thus, central administration of DMSO in the relatively high concentrations required to solubilize various drugs appears to be a suitable vehicle for food intake studies, whereas peripheral administration of these concentrations of DMSO is not recommended.

Similar findings have also been reported by those using DMSO as a vehicle on interleukin-1β (IL-1β)-induced fever in rats, regulation of body temperature and memory. Unilateral injections of platelet-activating factor (mc-PAF) into the hippocampus (0.5 μl, 50% DMSO) showed an enhanced effect on memory but there was no effect following a structurally similar analog of mc-PAF (lyso-PAF), suggesting that the effect of mc-PAF was not due to alteration in the integrity of the neurons as a consequence of using DMSO (Teather et al., 1998). Bilateral injections of 25% DMSO into the medial preoptic area (0.2 μl) were found in another study not to produce any changes in the sleep–wakefulness pattern or any changes to rectal temperature (Ramesh and Kumar, 1998), except within the first 15 min. The authors conclude that this is a nonspecific effect from animal handling based on the similar changes in temperature following the administration of DMSO, saline, yohimbine and clonidine. Intracerebroventricular injections of the EP_1 antagonist SC19220 (3 μl, 100% DMSO) attenuated the fever induced by IL-1β (Oka et al., 1998). If a vehicle is producing a nonspecific effect, in some cases, it masks any expected changes from the drug, but that was not evident from this study. One study determined that injections of DMSO into the dorsal raphe or substantia nigra (1 μl) resulted in a stronger effect on

EEG activity within the first 5 min (not evident by 10 min) within the dorsal raphe than in the substantia nigra. No comparison vehicle was used in this study and the authors were not clear as to the cause of this unless the responses to stress within these two brain sites differed or DMSO in this volume and concentration produced alterations in serotonin activity (Wala et al., 1997).

Although peripheral administration of DMSO (concentrations greater or equal to 60%) is known to increase the penetrability of substances across the skin following topical application (Horita and Weber, 1964; Klingman, 1965a,b; Stoughton and Fritsch, 1964), DMSO has been shown not to enter actual membranes such as those of the blood–brain barrier or skeletal muscle. Therefore, the concern about the potential effects of DMSO on the integrity of neurons may not be justified. Following intraperitoneal injection, DMSO (100%) was not found to alter the permeability of the blood–brain barrier or that of skeletal muscle, suggesting that although DMSO might enable the transit of other substances across biological membranes such as the blood–brain barrier, DMSO itself does not seem to penetrate such barriers. Furthermore, ^{35}S-DMSO is reported to accumulate along the exterior border of the cell and it has been suggested that all membranes are relatively impermeable to even high doses (Malinin et al., 1969). Two mechanisms have been proposed by which DMSO enhances the passage of substances across biological membranes. DMSO may associate itself with the penetrating molecule and enhance its lipophilicity (Csaky and Ho, 1966) or it may affect proteins of the membranes or enzymes associated with membrane transport (Friedman et al., 1969; Monder 1967). Thus, although it is not certain the extent to which DMSO in concentrations used in this study may either penetrate into the neurons or alter the integrity of the neurons within the PVN, it did not appear to affect measureable behavioral outputs such as food intake over the course of study.

In summary, PVN injection of NE stimulated food intake similarly when administered in aCSF or 75% DMSO vehicle. Although there are potential complications with using DMSO in other situations, DMSO in relatively high concentrations, up to 75%, appears to be an adequate vehicle for the administration of NE (and likely other water insoluble substances) in small volumes (0.3 μl) into specific brain regions. Because of the potential complications with using DMSO, caution should be exercised when testing the effects of high concentrations of DMSO in the brain on various parameters and alternative vehicles (aCSF) should be used as a comparison whenever possible.

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