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2-Deoxy-D-Glucose Analgesia: Influences of Opiate and Non-Opiate Factors

RICHARD J. BODNAR, DENNIS D. KELLY AND MURRAY GLUSMAN

Department of Behavioral Physiology, New York State Psychiatric Institute and Department of Psychiatry, 722 West 168th Street, New York, NY 10032

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BODNAR, R. J., D. D. KELLY AND M. GLUSMAN. *2-deoxy-D-glucose analgesia: influences of opiate and non-opiate factors*. PHARMAC. BIOCHEM. BEHAV. 11(3) 297–301, 1979.—Acute administration of 2-deoxy-D-glucose (2-DG), an antimetabolic glucose analogue induces a powerful analgesia which adapts following repeated administration. 2-DG analgesia displays significant cross-tolerance with morphine , and like morphine analgesia, is potentiated in hypophysectomized rats. The present study examined further the role of opiates in 2-DG analgesia by examining whether the opiate antagonist, naloxone, would affect 2-DG analgesia, and whether ineffective doses of 2-DG and morphine would interact in a synergistic fashion to induce analgesia. Nociceptive thresholds were measured by the flinch-jump test. Naloxone doses of 1, 5, 10 and 20 mg/kg were all ineffective in reducing significantly 2-DG (600 mg/kg) induced pain threshold elevations. Naloxone failed to attenuate 2-DG (350 mg/kg) analgesia whether administered before or after the 2-DG injection. On the other hand, simultaneous administration of sub-analgesic doses of 2-DG (200 mg/kg) and morphine (2.5 mg/kg) summated to produce significant analgesia. Thus, 2-DG analgesia is similar to opiates in its tolerant and summative actions, yet dissimilar in that naloxone is ineffective in reversing its effects.

Analgesia Pain 2-Deoxy-D-Glucose Morphine Naloxone Synergy Stress Rats

ACUTE administration of the anti-metabolic glucose analogue [43] and glucoprivic stressor [16, 26, 43], 2-deoxy-D-glucose (2-DG) has been shown to induce a powerful, transient analgesia as measured by both operant and reflex pain tests [12,13]. Repeated daily injections of 2-DG result in a behavioral adaptation to the analgesia [12] in much the same way that the analgesic effects of other stressors such as cold-water swims (CWS) [8, 10, 32] and inescapable foot shock (FS) [1, 17, 24, 33] dissipate with chronic exposure [1, 11, 33]. 2-DG analgesia is apparently related to CWS analgesia since full and reciprocal cross-tolerance develops between these two stressors [42]. Despite this similarity, the interactions of 2-DG and CWS with morphine differ. While CWS and morphine analgesia fail to develop cross-tolerance [15,31], 2-DG and morphine analgesia do. Morphine-tolerant rats are insensitive to 2-DG's analgesic properties, and morphine analgesia is attenuated in rats pretreated with 2-DG [42]. While CWS, FS and immobilization analgesia are attenuated by hypophysectomy [4,7], Reference Note [2], 2-DG analgesia is enhanced by removal of the pituitary [6].

Other data suggest that stress-induced and opiate analgesia may differ in their modes of action. Neither CWS, FS nor immobilization analgesia can be reversed by high doses of the potent opiate antagonist, naloxone [1, 3, 9, 10, 14, 24]. Spinal dorsolateral funiculus lesions which attenuate morphine analgesia [5,25] fail to alter FS analgesia [25]. Finally, while increases in brain opiate receptor activity and decreases in brain ^{3}H-leu-enkephalin activity occur following FS [1, 18, 19, 33], ^{3}H-met-enkephalin activity is unaltered [22] and hypothalamic ^{3}H-leu-enkephalin activity decrease following this stressor [40].

The present set of experiments examined further the relationship between 2-DG and morphine analgesia. The first experiment investigated the effects of naloxone upon 2-DG analgesia across a wide dose range (1, 5, 10 and 20 mg/kg). The second experiment examined the temporal parameters of naloxone and 2-DG administration to determine whether naloxone might exert different effects if administered before the onset of glucoprivation. The third experiment determined whether low, sub-analgesic doses of 2-DG might interact with similarly subanalgesic doses of morphine so as to produce significant analgesia.

EXPERIMENT 1: 2-DG ANALGESIA AND NALOXONE DOSE

METHOD

Eight male albino Sprague-Dawley rats (300–450 g) were tested for flinch-jump thresholds using a modification of the Evans procedure [21]. Electric shocks were delivered through a 30-cm floor composed of 14 grids by a 60-Hz constant current shock generator and grid scrambler. Using an ascending method of limits of successively more intense shocks, the flinch threshold was defined in mA as the lowest intensity that elicited a withdrawal of a single paw from the grids. The initial-jump threshold was defined as the lowest intensity that elicited simultaneous withdrawal of both hindpaws from the grids. The jump threshold was defined as the lowest of two consecutive intensities that elicited a jump as above. Each trial began with the animal receiving a 300-msec foot shock at a current intensity of 0.1 mA. Subsequent shocks occurred at 10-sec intervals and were increased in

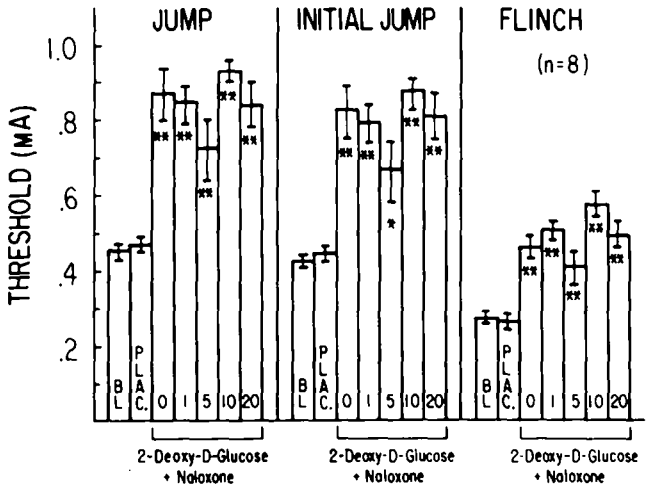

FIG. 1. Alterations in flinch-jump thresholds following administration of 2-deoxy-D-glucose (600mg/kg) alone and paired with 1, 5, 10 and 20 mg/kg doses of naloxone. *: $p<0.05$; **: $p<0.01$.

equal 0.05 mA steps until all three nociceptive thresholds were determined. After each trial, the current intensity was reset to 0.1 mA for the next trial until 6 trials were completed. Daily flinch, initial-jump and jump thresholds were each computed as the mean of these 6 trials. Stable baseline flinch-jump thresholds were verified over 3 days.

Each rat was then placed on a 2-session per week schedule for 5 weeks. Each pair of sessions occurred on successive days, the first always programmed as a placebo or control day, the second as an experimental drug session. On the weekly drug days, animals were injected intraperitoneally with 2-DG (600 mg 2-DG/2ml sterile water/kg body weight) 60 min before flinch-jump testing. Five min prior to this test, each rat received a subcutaneous injection of naloxone (Naloxone Hydrochloride, Endo Labs/1 ml sterile water) at one of five doses: 0, 1, 5, 10 or 20 mg/kg. The order in which the doses of naloxone were administered was determined by random placement of animals in an incomplete counterbalanced design such that each rat received all 5 doses of naloxone in conjunction with the five 2-DG injections during the 5-week paradigm. On the paired control days, placebo injections of similar volumes to the test injections were administered both 60 min and 5 min prior to flinch-jump testing. The experimenter conducting the flinch-jump test was uninformed of the specific experimental conditions.

RESULTS

Figure 1 displays the mean elevations over baseline thresholds induced by 2-DG injections in conjunction with the various doses of naloxone. No significant differences among the five placebo conditions were noted for any nociceptive measure, indicating that the animal's pain thresholds returned to normal following each experimental manipulation. Therefore, a pooled placebo mean is denoted. As noted with asterisks on figure 1, correlated difference score t-tests between each experimental pairing with its corresponding placebo control indicated that 2-DG induced significant analgesia regardless of the dose of naloxone with which it was paired. Furthermore, separate, repeated-measures analyses of variance comparing the five 2-DG/naloxone (0, 1, 5, 10, 20 mg/kg) conditions revealed no significant dose-related differences for jump, $F(4,28)=2.09$, and initial-jump, $F=2.18$, thresholds, but indicated significant effects across subjects on each measure, jump: $F(4,28)=3.18$, $p<0.05$, initial-jump: $F=3.38$, $p<0.05$.

EXPERIMENT 2: 2-DG ANALGESIA AND NALOXONE PRE- OR POST-TREATMENT.

The first experiment revealed that naloxone, administered 5 min before nociceptive testing but after 2-DG injection, had no effect upon 2-DG analgesia across a wide dose range. Thus, naloxone appears unable to reverse an analgesic state already established by 2-DG. One possible mechanism by which 2-DG may exert its analgesic effect is through activation of intrinsic pain-inhibitory processes during the stressful onset of glucoprivation. If so, then naloxone administered after 2-DG injections would not be present to block this activation. Therefore this experiment examined whether naloxone would exert an antagonistic effect upon 2-DG analgesia if it was administered before the 2-DG injection.

METHOD

Nine additional naive male rats were tested for flinch-jump thresholds in the manner described in Experiment 1. Each rat was exposed to six experimental conditions according to an incomplete counterbalanced design. In the first (SAL/SAL) condition, rats were injected with a placebo (1 ml sterile water/kg body weight) 30 min (IP) and 5 min (SC) before flinch-jump testing. In the second (NAL/SAL) and third (SAL/NAL) conditions, rats received the intraperitoneal placebo injection 30 min prior to testing and naloxone (10 mg/ml sterile water/kg body weight, SC) either 35 min or 5 min prior to testing. In the fourth (2-DG/SAL) condition, rats received a 350 mg/kg dose of 2-DG (300 mg 2-DG/ml sterile water/kg body weight, IP) 30 min prior to testing, and a subcutaneous placebo injection 5 min prior to testing. In the fifth and sixth conditions, rats received the 2-DG injection 30 min prior to testing and the naloxone injection either 35 min (NAL/2-DG) or 5 min (2-DG/NAL) prior to testing. Tests were conducted twice each week over a 3-week span. Subsequently, each animal received morphine (10 mg morphine sulfate/ml buffered solution/kg body weight, SC) 30 min prior to testing and either placebo (MOR/SAL) or a dose of 10 mg/kg of naloxone (MOR/NAL) 5 min prior to testing. Half of the animals were exposed first to the latter condition while remainder were in the reverse sequence.

RESULTS

Figure 2 shows that naloxone failed to attenuate 2-DG analgesia whether administered before or after 2-DG. Separate, repeated-measures analyses of variance revealed significant alterations in all nociceptive measures across both experimental conditions, jump: $F(7,56)=25.00$, $p<0.01$; initial-jump: $F=20.33$, $p<0.01$; flinch: $F=6.96$, $p<0.01$, and subjects, jump: $F(8,56)=6.33$, $p<0.01$; initial-jump: $F=5.67$, $p<0.01$; flinch: $F=4.11$, $p<0.01$. Post-Hoc Scheffe comparisons indicated that; (a) jump and initial-jump thresholds were increased significantly following 2-DG injections whether or not these were paired with naloxone, (b) neither pre- nor post-treatment with naloxone significantly altered 2-DG analgesia, (c) consistent with previous reports [9, 14, 20, 23], naloxone itself failed to alter jump thresholds, and (d) as reported elsewhere [21] flinch thresholds did not co-vary

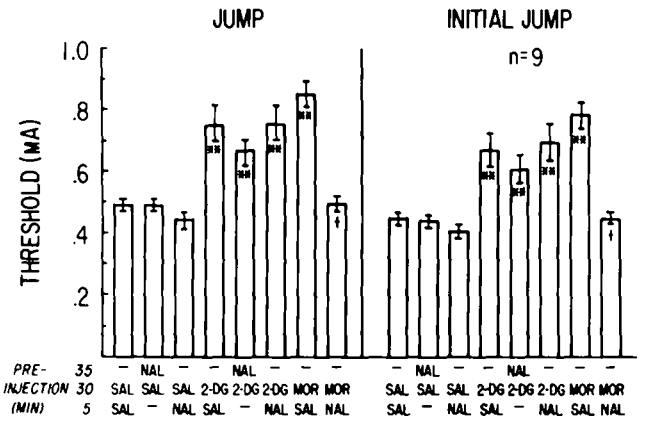

FIG. 2. Alterations in jump measures (±S.E.M.) following either administration of 2-deoxy-D-glucose (2-DG: 350 mg/kg) or morphine (MOR: 10 mg/kg) alone and paired with pre-treatment or post-treatment with naloxone (NAL: 10 mg/kg). **: different from baseline ($p<0.01$); +: different from MOR/SAL ($p<0.01$).

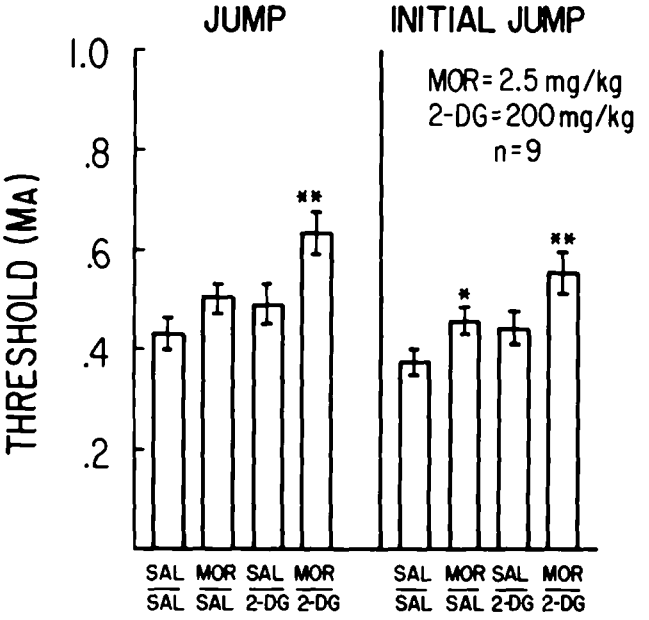

FIG. 3. Summative interaction in jump measures (±S.E.M.) following paired injections of 2-deoxy-D-glucose (2-DG) and morphine (MOR). *: $p<0.05$; **: $p<0.01$.

with jump measures in response to the pharmacological manipulations. Since the same dose of naloxone totally reversed morphine analgesia (10 mg/kg,) it is unlikely that its lack of effect upon 2-DG analgesia could be attributed to any non-specific factor.

EXPERIMENT 3: 2-DG AND OPIATE ANALGESIA: INTERACTION AND SYNERGY

The first two experiments showed that naloxone over a wide range failed to attenuate 2-DG analgesia and that this ineffectiveness was not related to the temporal order of naloxone and 2-DG administration. However, 2-DG and morphine analgesia have been shown to share the common properties of at least partial cross-tolerance [42] and of sensitivity to hypophysectomy [6, 27, 37]. Consequently, to explore further the relation of 2-DG to opiate analgesia, this experiment investigated whether administration of sub-analgesic doses of both agents might summate or synergize to produce analgesia.

METHOD

Nine additional naive male rats were tested for flinch-jump thresholds as described in Experiment 1. Each rat was exposed to four experimental conditions according to an incomplete counterbalanced design. All injections occurred 30 min prior to flinch-jump testing. In the first (SAL/SAL) condition, rats received two placebo (1 ml sterile water/kg body weight) injections, one intraperitoneal and one subcutaneous. In the second (MOR/SAL) condition, rats received both a placebo injection (IP) and morphine (2.5 mg morphine sulfate/ml buffered solution/kg body weight, SC). In the third (SAL/2-DG) condition, rats received a placebo injection (SC) in conjunction with 2-DG (200mg 2-DG/ml sterile water/kg body weight, IP). In the fourth (MOR/2-DG) condition, rats received a subcutaneous morphine injection in conjunction with an intraperitoneal 2-DG injection.

RESULTS

Figure 3 illustrates that while individual administration of either morphine or 2-DG had only slight antinociceptive effects, administration of both significantly elevated jump thresholds. Separate, repeated-measures analyses of variance revealed significant alterations across experimental conditions in jump, $F(3,23)=9.29$, $p<0.01$, initial-jump, $F=9.04$, $p<0.01$, and flinch, $F=69.00$, $p<0.01$ thresholds. Significant inter-subject variability was noted for jump, $F(8,24)=3.43$, $p<0.01$, and initial jump, $F=4.05$, $p<0.01$, measures, but not for the flinch ($F=1.67$) measure. As noted by asterisks in Fig. 3, *a posteriori* Scheffe comparisons indicated that 200 mg/kg of 2-DG induced only slight, non-significant increases in both jump measures, while morphine alone at 2.5 mg/kg induced a small, yet significant increase in initial-jump thresholds. Like 2-DG, it had no significant effect upon jump thresholds. When the two sub-analgesic doses of 2-DG and of morphine were paired, significant increases in both jump measures were observed. To ascertain for each subject whether this observed interaction was additive or multiplicative, the SAL/SAL placebo value was subtracted successively from the morphine alone (MOR/SAL), 2-DG alone (SAL/2-DG) and the combined (MOR/2-DG) conditions. Then the difference scores for the MOR/SAL and the SAL/2-DG conditions were combined for each animal and compared with the corresponding difference score for the MOR/2-DG condition. Separate correlated difference score t-tests for each nociceptive measure revealed that the summed analgesia of the MOR/SAL and SAL/2-DG conditions was not significantly different from the analgesia of the MOR/2-DG condition for jump, $t(8)=1.13$, or initial-jump, $t=0.45$, measures, suggesting that the interaction of 2-DG and opiate analgesia was additive.

DISCUSSION

The results of the present study suggest that, unlike morphine analgesia [34], 2-DG analgesia is unaffected by the

opiate antagonist, naloxone. While capable of fully eliminating morphine analgesia at doses as low as 0.5 mg/kg, naloxone failed to affect 2-DG analgesia across a range of doses from 1 to 20 mg/kg. Naloxone failed to affect 2-DG analgesia whether administered before or after glucoprivic stress was induced, suggesting that 2-DG analgesia is not mediated by an endogenous opiate-like system. Still, sub-analgesic doses of 2-DG and morphine when administered concomitantly did interact, or sum, to produce significant analgesia. These seemingly paradoxical results are similar to opiate interactions with analgesia induced by electrical stimulation and by chlordiazepoxide. Like 2-DG analgesia [42], stimulation-produced analgesia exhibits both analgesic synergy and partial cross-tolerance with morphine [30, 35, 41]. However, although some studies [2, 30, 38] report that stimulation-produced analgesia is either fully or partially reversed by naloxone, others [39,45] indicate that the opiate antagonist is ineffective. Chlordiazepoxide, which induces significant analgesia on both operant and reflex pain measures [28], exhibits partial cross-tolerence with morphine (Reference Note 1). Yet high doses of naloxone are ineffective in reversing chlordiazepoxide analgesia on the flinch-jump test and only partially effective on the operant liminal escape test [29].

Recently, several investigators [9, 15, 24 36] have postulated the existence of at least two independent branches of an intrinsic pain-inhibitory system. One of these branches seems to be opiate and activated by morphine, while the other seems to be non-opiate and activated by such stressors as CWS, FS and insulin. The existence of a non-opiate branch of pain-inhibition is substantiated by the observations that (a) CWS and morphine fail to exhibit analgesic cross-tolerance [15,31]; (b) CWS, FS and immobilization analgesia are not reversed by high doses of naloxone [1, 4, 9, 10, 14, 24]; (c) dorsolateral funiculus spinal cord lesions attenuated morphine [5,25], but not FS [25] analgesia; and (d) hypophysectomy potentiates analgesia induced by high doses of morphine [6, 27, 37] while attenuating CWS, FS immobilization and insulin analgesia [4, 6, 7] Reference Note [2]. This latter finding, in addition to morphine microintion studies (see review; [44]), indicates that the opiate branch of pain-inhibition is centrally-mediated while the proposed non-opiate branch possesses some peripheral components. The results of the present and prior experiments suggest that 2-DG may induce analgesia by acting upon both branches of the body's pain inhibitory system. 2-DG analgesia clearly shares some characteristics of opiate analgesia, since it exhibits analgesic cross-tolerance with morphine [42], is potentiated by hypophysectomy [6] and can sum with sub-analgesic morphine levels to produce analgesia. On the other hand, 2-DG analgesia also has some non-opiate properties since it exhibits full and reciprocal cross-tolerance with CWS [42] and as demonstrated in the present study, it is not reversed by naloxone.

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