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# Differential Inhibition by Propranolol of Feeding Induced in Rats by Various Stimuli

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BRYANT, H. U., P. V. MALVEN AND G. K. W. YIM. *Differential inhibition by propranolol of feeding induced in rats by various stimuli.* PHARMACOL BIOCHEM BEHAV 21(4)651–654, 1984.—Opiate receptor blockade, or forced imbibition of 2% NaCl to deplete pituitary dynorphin decreases 2-deoxy-D-glucose (2-DG), but not insulin-induced hyperphagia, indicating a possible role for dynorphin in the cating associated with endogenous opiates. Beta-adrenergic receptor blockade decreases vasopressin release induced by 2-DG but not by insulin. Because vasopressin and dynorphin are sometimes co-localized, it was hypothesized that naloxone-sensitive feeding might be selectively inhibited by beta-adrenergic blockade with propranolol. Propranolol in doses as low as 2.5 mg/kg inhibited 4 hr feeding induced by 2-DG (400 mg/kg). Propranolol did not significantly affect feeding induced by ketocyclazocine administration (3.0 mg/kg) or by 24 hr food deprivation. Feeding stimulated by insulin (10 U/kg) was significantly inhibited by propranolol (2.5 mg/kg) only when the propranolol was reinjected during the period 2 hr after insulin injection, when the induced feeding was greatest. In summary, propranolol inhibited opiate-related (2-DG) as well as opiate-independent (insulin) hyperphagias. It also failed to inhibit food intake resulting from the opiate related stimulus of 24 hr food deprivation. Therefore, naloxone sensitive hyperphagias were not specifically inhibited by beta-adrenergic blockade, indicating that vasopressin-associated dynorphin is not involved in opiate related feeding.

Dynorphin Feeding Beta-adrenergic blockade

BOTH 2-deoxy-D-glucose (2-DG) and insulin stimulate feeding in rats, but different mechanisms may be involved. Opiate antagonists such as naloxone readily depress 2-DG hyperphagia [6], while insulin induced feeding is apparently not as sensitive to opiate receptor blockade by naloxone. Naloxone reduced the food intake of insulin injected rats when measured at 1 hr [13] but not at 3 hr [6]. Insulin-induced feeding may involve both opiate and non-opiate components [16]. Recent studies suggest that the opiate receptors involved in feeding are likely of the mu and/or kappa type [4, 7, 10, 14, 15, 18]. Since the kappa type endogenous opioid, dynorphin, is a powerful appetite stimulant [9], we recently examined the feeding behavior of rats drinking 2% NaCl, a regimen that depletes dynorphin levels in the neurointermediate lobe of the pituitary [5]. Feeding induced by 2-DG was reduced, whereas feeding subsequent to insulin or ketocyclazocine, a kappa receptor agonist, was intact [19,20]. The present experiment further explores the possible role of dynorphin in feeding.

In addition to their hyperphagic activity, both 2-DG and insulin stimulate vasopressin release from the neurohypophysis. However, they appear to release vasopressin by different mechanisms since administration of the beta-adrenergic blocker, propranolol, blocked 2-DG induced increases in plasma vasopressin but not those induced by insulin [1,2]. Vasopressin and dynorphin co-exist in the nerve terminals of the neurohypophysis, and in some (e.g., hypothalamic), but not all, neurons of the brain [8,18]. The primary hypothesis was that 2-DG stimulated the release of dynorphin, along with vasopressin, by a beta-adrenergic mechanism. The dynorphin, in turn, then could account for the naloxone reversible hyperphagia following 2-DG administration.

According to the hypothesis, 2-DG induced feeding should be antagonized by propranolol pretreatment, while insulin hyperphagia should not be affected. Also, feeding induced by direct kappa receptor activation with agents such as ketocyclazocine should not be reduced by propranolol since the proposed beta-adrenergic mechanism occurs prior to kappa receptor activation in the hypothesis. The effect of propranolol on the naloxone sensitive feeding induced by 24 hr food deprivation was also studied.

## METHOD

Male, Sprague-Dawley rats (300–400 g) were housed individually in metal cages (25×21×20 cm) at least 10 days prior to testing. Animals were handled and given saline injections in four preliminary sessions to habituate them to the experimental procedures. The animals had free access to water and Wayne Lab Blox placed on the cage floor. Illumination was on a 12/12 hr schedule with light onset at 8:00 a.m. Room temperature was maintained at 23–26°C.

Daytime 4 hr food intake was stimulated by SC injection of 2-DG (400 mg/kg), insulin (10 U/kg), ketocyclazocine (3.0

mg/kg) or by 24 hr food deprivation, 3 hr after light onset when food intake is low. Immediately after injection the rat was returned to its home cage (food deprivation also occurred in the home cage) and food intake was measured to the nearest 0.1 g by subtracting spillage on paper towels and uneaten food from a premeasured supply. Water was available during the study ad lib. Experimental groups consisted of at least six randomly assigned rats.

Propranolol was administered in a dosage range of 2.5 to 10 mg/kg, 20 min prior to the hyperphagic stimulus (e.g., 2-DG injection) and subsequent presentation of the food. Control rats received the appropriate vehicle.

Since there was some concern over the short half-life of propranolol in the plasma (about 60 min [3]), and the longer time required for full development of insulin hyperphagia (about 4 to 6 hr), a second insulin/propranolol study was performed in order to achieve concurrence of the peak effects of both drugs. Food was withheld for 2 hr after propranolol pre-treatment and the insulin injection. A second dose of propranolol was injected and food intake was then measured at 1, 3 and 4 hr following presentation of the food.

Sources of drugs were: 2-DG from U.S. Biochemical Co. (Cleveland, OH); insulin (Iletin U-100) from Eli Lilly and Co.; propranolol from Sigma Chemical Co.; and ketocyclazocine from Sterling-Winthrop Research Institute. Propranolol, 2-DG and insulin solutions were made fresh by dissolving them in 0.9% saline just prior to injection. Ketocyclazocine was dissolved in distilled water with an equimolar amount of HCl, and back titrated with NaOH to a pH of 4.5, just prior to injection. Ketocyclazocine remained in solution at this pH. Injections were given SC in volumes of 1.0 ml/kg.

Unless otherwise specified, all results were compared using analysis of variance and post-hoc Newman-Keuls tests where indicated.

## RESULTS

Propranolol at doses of 2.5 and 5.0 mg/kg significantly ($p<0.01$) depressed 4 hr food intake induced by 2-DG (Fig. 1A). Following 2-DG injection, the saline pretreated animals responded with a mean 4 hr intake of 8.0±0.8 g, while the 2.5 and 5.0 mg/kg propranolol pretreated animals only ate 4.6±0.7 g (reduction of 42%) and 4.0±0.6 g (reduction of 49%) respectively. The majority of 2-DG induced feeding occurred in the first 2 hr period, and the attenuation of 2-DG hyperphagia by propranolol was evident at this time point as well.

When tested in the same manner as with 2-DG (i.e., food presentation immediately following the feeding stimulus), there was no significant effect of propranolol on 4 hr insulin induced food intake (Fig. 1B). Compared with 2-DG, the insulin feeding stimulus caused a slower onset of feeding, and food intake during the first 2 hr after insulin was relatively low (not shown). This difference complicated the comparison of propranolol blockade of 2-DG and insulin induced feeding. Therefore, another experiment was conducted in which (1) food was withheld until 2 hr after insulin when the feeding stimulus was most effective, and (2) the propranolol injection was repeated just before a 1 hr presentation of food. Using this paradigm, propranolol at a dose per injection of 2.5 mg/kg reduced 1 hr food intake by 44% (Fig. 2). Propranolol doses of 5.0 and 10.0 mg/kg produced similar reductions in 1 hr food intake. The rats in this study were allowed to eat for an additional 3 hr in order to measure 4 hr

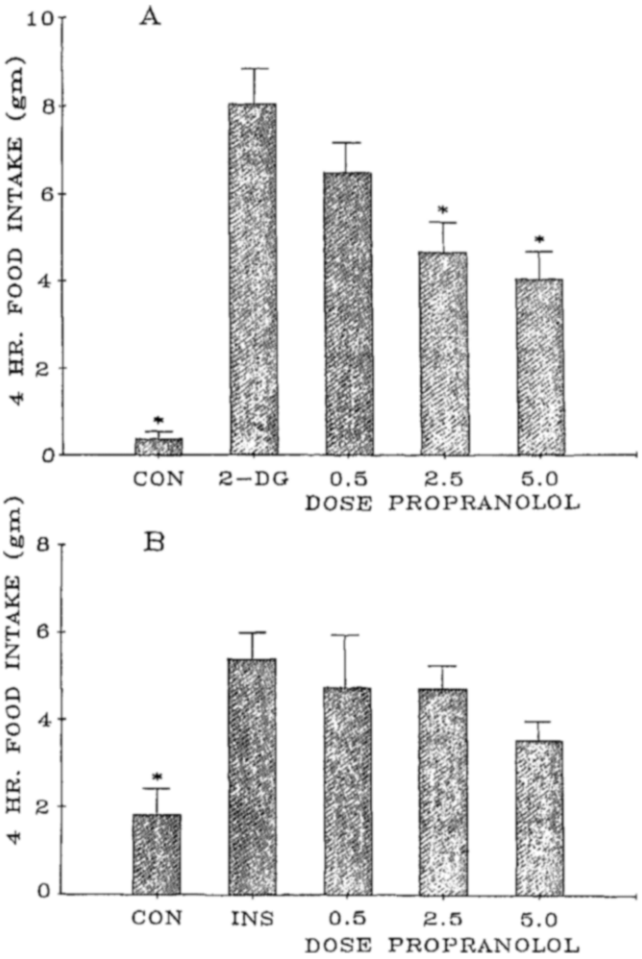


FIG. 1. Effect of various doses (in mg/kg) of propranolol (PROP) on daytime feeding elicited by 2-deoxy-D-glucose (2-DG, 400 mg/kg; panel A), insulin (INS, 10 U/kg; panel B) or saline (CON) injected SC to nondeprived animals. Bars represent mean 4 hr food intake (g) ±S.E. *=$p<0.01$ vs. 2-DG group.

intakes (data not shown). Two-way analysis of variance indicated a significant effect of propranolol treatment, $F(4,125)=9.753$, $p<0.01$. Subsequent Newman Keuls analysis of 4 hr intakes confirmed that propranolol significantly decreased insulin induced feeding, but intakes for the combined insulin-propranolol groups remained greater than saline controls.

The effects of propranolol on 4 hr feeding induced by the putative kappa opiate agonist, ketocyclazocine, are presented in Fig. 3A. Ketocyclazocine stimulated feeding, but the magnitude was less than that stimulated by 2-DG (Fig. 1A) or insulin (Fig. 1B). Propranolol tended to decrease feeding, but this effect was not significant even at the 10.0 mg/kg dose of propranolol. The feeding response to ketocyclazocine was rapid in onset, with most of the feeding occurring in the first 2 hr following injection of kappa agonist (similar to 2-DG). Food deprivation for 24 hr produced hyperphagia of the greatest magnitude with a rapid onset, which was not attenuated by propranolol treatment (Fig. 3B).

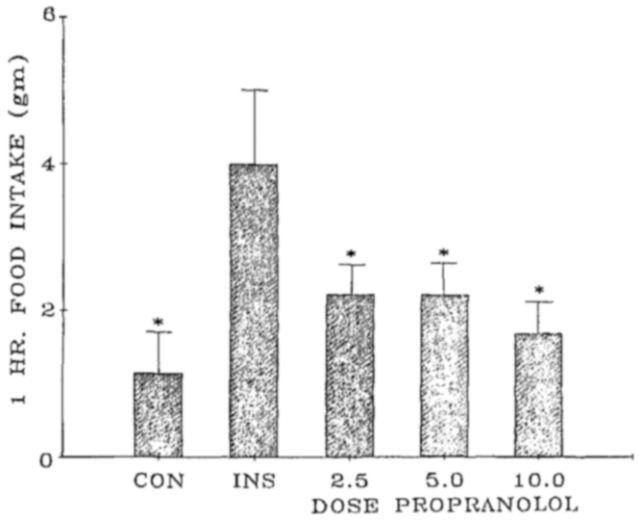


FIG. 2. First hr insulin (INS, 10 U/kg) or saline (CON) induced food intake following a 2 hr delay, and a second dose (in mg/kg) of propranolol (PROP) injected SC to previously non-deprived animals. Bars represent mean 1 hr food intake (g) ±S.E. *=$p<0.05$ vs. INS group.

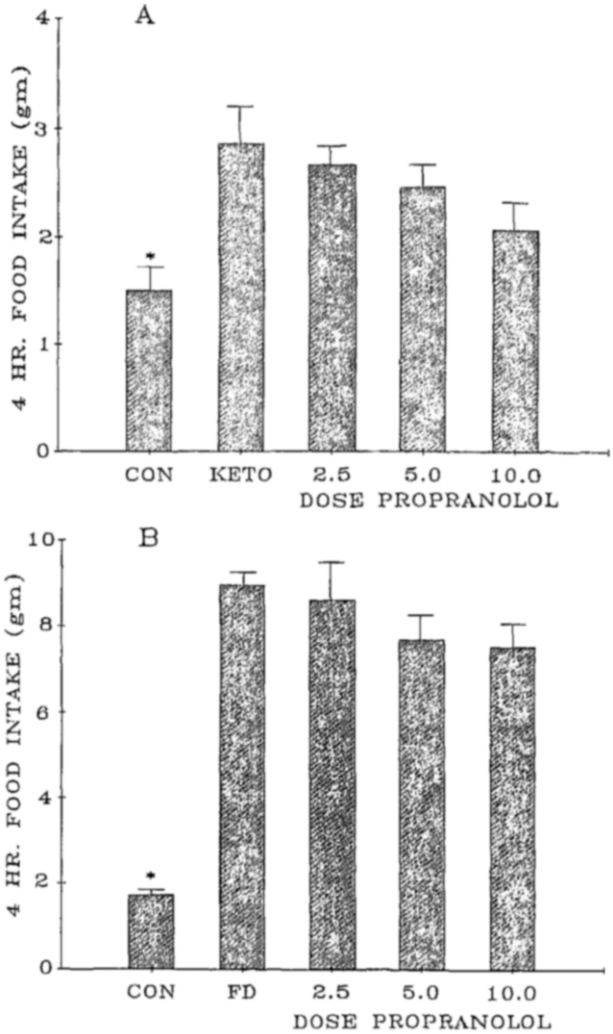


FIG. 3. Effect of various doses (in mg/kg) of propranolol (PROP) on daytime feeding elicited by ketocyclazocine (KETO, 3.0 mg/kg) and a pH 4.5 control (CON, panel A) or by 24 hr food deprivation (FD) and saline control (CON, panel B). Bars represent mean 4 hr food intake (g) ±S.E. *=$p<0.05$ vs. KETO group (A) or FD group (B).

## DISCUSSION

These experiments reveal an apparent beta-adrenergic mechanism involved in the hyperphagia subsequent to the SC injection of 2-DG. Peripheral administration of propranolol produced a significant inhibition of the feeding at the relatively low dose of 2.5 mg/kg. This is as expected since Baylis and Robertson described similar sensitivity to beta-blockade for 2-DG induced release of arginine-vasopressin [1,2]. Furthermore, the finding that propranolol did not significantly depress feeding after direct kappa receptor stimulation with ketocyclazocine was consistent with our hypothesis that the naloxone sensitive hyperphagia following 2-DG may be mediated by the endogenous kappa receptor ligand, dynorphin.

When propranolol was tested with the insulin and food deprivation stimuli, the results contradicted our hypothesis of beta-adrenergic mechanisms specific for naloxone sensitive feeding. The food deprivation stimulus, which produces naloxone sensitive hyperphagia [20], but does not alter tissue levels of dynorphin [12], was not inhibited by propranolol. These results emphasize that 2-DG and food deprivation hyperphagia are not completely equivalent even though they are both inhibited by naloxone. Interestingly, the naloxone reversible feeding induced by tail pinch stress is also not blocked by propranolol, but is reduced by central administration of isoproterenol, a beta-adrenergic agonist [11].

The ability of propranolol to inhibit insulin induced feeding depended upon the time of injection. When injected alone, 20 min prior to insulin, the 2.5 mg/kg dose of propranolol failed to inhibit 4 hr food intake, much of which occurred 2 to 4 hr after insulin (Fig. 1B). When injected a second time, 2 hr after insulin, and just before presentation of the food, the 2.5 mg/kg dose markedly inhibited insulin induced feeding (Fig. 2). Thus, one injection of propranolol could not sustain beta-adrenergic blockade until the major period of insulin hyperphagia which occurred 2 to 3 hr after injection. Furthermore, the ability of propranolol to inhibit insulin induced feeding contrasted with its inability to inhibit insulin induced release of vasopressin. This difference is consistent with dynorphin and vasopressin being differentially localized in brain areas other than the neurohypophyseal neurons. These results suggest that the vasopressin-linked dynorphin pools are not involved in opiate-related feeding. Finally, these findings indicate that plasma vasopressin cannot be used to monitor dynorphin release in opiate related hyperphagias.

## ACKNOWLEDGEMENTS

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