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Metabolic Modulation by Amino Acid Stimulation of the Paraventricular Nucleus of the Hypothalamus

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ATRENS, D. M. AND J. A. MENÉNDEZ. *Metabolic modulation by amino acid stimulation of the paraventricular nucleus of the hypothalamus.* PHARMACOL BIOCHEM BEHAV **46**(3) 617–622, 1993.—The role of the paraventricular nucleus of the hypothalamus (PVN) in the regulation of energy expenditure and energy substrate utilization was investigated after the injection of the excitatory amino acid D,L-homocysteic acid (DLH) or its vehicle. Male Wistar rats with chronic PVN cannulae were tested for 1 h with no food available in an open-circuit calorimeter. Whereas low (0.5 nmol), excitatory doses of DLH increased energy expenditure, the thermogenic effect became smaller and then vanished as the DLH dose was increased to inhibitory levels (7 and 50 nmol). None of these doses affected motor activity, indicating a primary thermogenic effect. The highest dose (100 nmol) increased energy expenditure, but this appeared to be secondary to increased locomotor activity. The increased locomotor activity produced by the highest dose of DLH constitutes the first demonstration of an activity effect induced by stimulating the PVN. However, this effect likely reflects the activation of neighboring areas. Only the 50 nmol dose of DLH increased respiratory quotient, indicating a shift toward the preferential utilization of carbohydrates as an energy substrate. These data complement our findings with neuropeptide Y and insulin in showing that different doses of the same substance injected into the PVN may produce qualitatively different effects. Furthermore, the present study demonstrates that exciting PVN neurons activates catabolic forces, whereas inhibiting them activates anabolic forces.

Paraventricular nucleus of the hypothalamus PVN Excitatory amino acid Homocysteic acid Metabolism Energy balance Thermogenesis Energy expenditure Respiratory quotient Energy substrate utilization Body weight Indirect calorimetry Obesity Rat

THE paraventricular nucleus of the hypothalamus (PVN) modulates feeding and body weight regulation (8,11,15,19). Recent studies from our laboratory have demonstrated that the PVN also modulates thermogenesis and energy substrate utilization (15–17,21). The latter metabolic effects respond differentially to numerous experimental manipulations and can be dissociated from changes in locomotor activity. These dissociations suggest that the metabolic and behavioural processes mediated by the PVN are subserved by distinct neural systems.

Whether PVN neurons excite or inhibit metabolism is still uncertain. Lesions of the PVN consistently increase food intake and produce obesity (8,19,22), but there is some question as to whether the reported increases in food intake are sufficient to produce the increases in body weight. If this were not the case, decreased thermogenesis would be implicated as a factor in the development of obesity.

Amir (1) has shown increased brown adipose tissue thermogenesis after the injection of the excitatory amino acid, glutamate, into the PVN. However, it is not clear whether such localised thermogenesis is sufficient to significantly alter overall energy balance. We have recently reported that norepinephrine and galanin injections into the PVN decrease whole-body thermogenesis (16,21), whereas insulin injections into the PVN increase thermogenesis (15). In contrast, neuropeptide Y injections into the PVN have no effect on thermogenesis (17). These findings suggest that the excitation and inhibition of thermogenesis mediated by the PVN may be subserved by distinct neural systems.

The modulation of energy expenditure by the PVN appears to be very different from its modulation of energy substrate utilization. This suggests differential neurochemical coding of these metabolic parameters. Further complexity of the the role of the PVN in metabolic modulation is indicated by the fact that different doses of the same substance injected into the PVN may enhance, inhibit, or have no effect on either metabolic parameter. We have demonstrated this effect with norepinephrine, neuropeptide Y, galanin, and insulin in the PVN

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as well as in the sulcal prefrontal cortex (15–17,21). We have also demonstrated this response complexity with D,L-homocysteic acid (DLH) injected into the sulcal prefrontal cortex.

The present study was designed to analyze the effects on energy expenditure and respiratory quotient of injections of DLH into the PVN. DLH is known for its selective depolarizing effect on neural perikarya (10). DLH is particularly interesting in the present context, because low doses (below about 5 nmol) produce excitation, whereas higher doses produce depolarization blockade and inhibition (10). Thus, DLH presents the opportunity to study excitatory and inhibitory effects at the same site.

METHOD

Subjects

Ten male Wistar rats obtained from the University of Sydney breeding farm weighed between 300 and 400 g at the time of surgery. The rats were individually housed in clear acrylic cages, with food (Allied Rat & Mouse Kubes, Sydney) and tap water provided ad lib. The colony room was maintained at 22 ± 2°C, with a 14L : 10D cycle. Every rat was handled daily for 1 week before surgery and for 2 weeks after to minimize the stress of human contact.

Surgery and Histology

The rats were anaesthetized with 1 ml/kg Ketalar (100 mg/ml ketamine hydrochloride; Parke-Davis Pty. Ltd.) and 0.1 ml Rompun (20 mg/ml xylazine hydrochloride; Bayer Australia Ltd.), both injected intramuscularly (IM). They were placed in a Kopf stereotaxic apparatus and implanted with a single, stainless steel, 22-ga guide cannula fitted with a dummy cannula (Plastics One, USA). The coordinates relative to bregma were: posterior 1.8, lateral 1.8, and ventral 7.2, with the cannulae being implanted at an angle of 10° off the midline (18). The tip of the cannula was aimed 1 mm dorsal to the PVN, and the injector cannula extended 1 mm beyond the tip of the guide cannula.

At the conclusion of testing, the rats were given a lethal dose of Nembutal, after which their brains were removed for histological analysis. The brains were frozen to −12°C, sectioned at 40 μm, and stained with toluidine blue O. Cannulae placements were determined microscopically with reference to the atlas of Paxinos and Watson (18).

Apparatus

Respiratory quotient (RQ) and energy expenditure (EE) were calculated after recording oxygen (O_2) consumption and carbon dioxide (CO_2) production in an open-circuit calorimeter. Two clear acrylic cylindrical chambers with stainless steel grid floors and a volume of 6.28 l each were used. One was used for testing the rats and the other as a reference standard for calibration of the atmospheric air. Compressed atmospheric air at a flow rate of 1600 ml/min and a pressure of 8 kPa above atmospheric was continuously drawn through both chambers. Drawing the air from a cylinder in which a large volume of air has been compressed eliminates local variability in both O_2 and CO_2 and ensures that the the composition of the air going into the chambers remains constant. A system of solenoids allowed the air leaving the test chamber to be split, and an aliquot was directed for analysis, while the air from the other chamber was exhausted to the atmosphere. A sample of 110 ml/min was directed through a Perma Pure (Toms River, NJ) permeation drier (model PD750-12PP), a CD-3A CO_2 analyzer, and a S-3A O_2 analyzer (Applied Electrochemistry, USA). The rest of the air was exhausted to the room. The analyzers were calibrated daily with primary gravimetric standards obtained from Commonwealth Industrial Gases, Sydney. Pre- and postexperimental analysis of the gas in the reference cage was conducted to evaluate any possible drift in the analyzers.

Motor activity was recorded by placing the testing chamber on an electronic balance (Mettler PE-2000) and using the unintegrated signal from the strain gauge. The reliability and validity of this method has been demonstrated in a number of other studies (12–17,21).

A microcomputer system controlled and monitored the calorimeter. The computer provided minute-by-minute records of air flow, CO_2 production, O_2 consumption, and activity counts. The following calculations were made: EE (kJ) = mole O_2 (364 + 113 RQ); RQ = vol. CO_2 produced/vol. O_2 consumed (3,4,6). Energy expenditure was expressed in J/g to account for different body weights.

Experimental Procedure

Each rat was habituated to the metabolic apparatus in 60-min tests before and after surgery. This procedure also provided baseline data on the metabolic and activity parameters and allowed the determination of any effects of the surgical procedure itself. The rats were also habituated to the injection procedure by introducing the injector cannula into the guide cannula on two separate occasions before any experimentation.

The experiment itself began approximately 2 weeks after surgery. The test sessions were conducted in the light phase of the cycle (between 10:00 a.m. and 4:00 p.m.). The rats were given a 30-min run in the testing chamber before each treatment. They were then removed and injected in a counterbalanced order with 0.30 μl of either sterile saline (NaCl 0.9%) or one of four doses of DLH (Sigma, USA): 0.5, 7, 50, and 100 nmol, dissolved in sterile saline. The pH of each injection solution was adjusted to 7.4 with 1 M NaOH. The doses were selected on the basis of DLH's effects on the sulcal prefrontal cortex (13), as well as the intrinsic characteristics of its excitation effect (10). The dummy cannula was removed, and the injections were performed over a 1-min period through a 28-ga injector (Plastics One) that projected 1 mm beyond the guide cannula. The rats were unrestrained during the injection procedure. After the injection, the injector cannula was removed and the dummy cannula resecured. The rats were then placed in the testing chamber, and respiratory exchange and activity were monitored for 60 min, after which they were returned to their home cages. Each test session in the calorimeter was preceded and followed by a 5-min analysis of air leaving the calibration chamber to assess any drift in the analyzers. In all cases, the pre/post tests indicated minimal analyzer drift and no data had to be discarded.

Food was not available during the test sessions and at least 7 days elapsed between successive injections in the same rat.

Data Analysis

The data from each DLH treatment were compared with those of the saline treatment by a two-way analysis of variance (ANOVA) with repeated measures on the treatment and time factors. The level of significance was set at 0.05.

RESULTS

No statistically significant differences were found between the presurgery, postsurgery, and saline injection data for energy expenditure, respiratory quotient, or locomotor activity. The values overlapped extensively and varied from: mean energy expenditure, 0.22–0.32 J/g; mean RQ, 0.83–0.90; total activity counts, 191–516. These data rule out surgically induced or saline-induced metabolic effects as significant mediators of the observed treatment effects. They also eliminate the possibility of any confounding due to simple volume injected or any cannula-induced damage to the PVN. The possibility of confounding due to cannula-induced tissue damage was also reduced by the fact that the histological analysis showed minimal incidental damage. The cannulae tracts themselves were closely grouped about 1 mm dorsal to the PVN over an anterior posterior range of 0.8 mm.

The 0.5 and 100 nmol doses of DLH respectively increased energy expenditure by an average of 34.4%, $F(1, 9) = 44.85$, $p < 0.001$, and 40.6%, $F(1, 9) = 50.51$, $p < 0.001$ (Fig. 1). The 7 and 50 nmol doses of DLH did not produce significant treatment effect on energy expenditure [$F(1, 9) = 0.01$ and 0.78, $p > 0.5$, respectively]. However, the 7 nmol dose did produce a significant treatment by time interaction, $F(60, 540) = 2.06$, $p < 0.001$, which reflects the initial small increase followed by a similar magnitude decrease toward the end of the session. The 50 nmol dose did not produce a significant treatment by time interaction, $F(60, 540) = 0.76$, $p > 0.05$. The main effect of time on energy expenditure was significant for all four doses of DLH [$F(60, 540) = 47.71$, 12.96, 8.95, and 19.84, $p < 0.001$, respectively] (see below for explanation).

There was no significant main effect of DLH treatment on respiratory quotient [$F(1, 9) = 0.25$, 0.03, 3.58, and 0.08, $p > 0.05$, respectively, for the 0.5, 7, 50, and 100 nmol doses] (Fig. 2). However, the 50 nmol dose of DLH did produce a significant treatment by time interaction, $F(60, 540) = 1.65$, $p < 0.01$, which reflects the increased respiratory quotient (32% average) produced by the 50 nmol dose of DLH, particularly toward the middle of the session (Fig. 2). The main effect of time on respiratory quotient was significant for all four doses of DLH [$F(60, 540) = 5.16$, 2.93, 6.17, and 11.26, $p < 0.001$, respectively] (see below for explanation).

There was no significant main effect of DLH treatment on activity [$F(1, 9) = 0.07$, 1.55, 0.01, and 1.80, $p > 0.05$, respectively, for the 0.5, 7, 50, and 100 nmol doses] (Fig. 3). However, the 100 nmol dose of DLH did produce a significant treatment by time interaction, $F(60, 540) = 1.99$, $p < 0.01$, which reflects the increased activity (34.1% average) produced by the 100 nmol dose of DLH in the first half of the session (Fig. 3). None of the other interactions was siginificant. The main effect of time on activity was significant for all four doses of DLH [$F(60, 540) = 3.21$, 3.36, 2.53, and 7.21, $p < 0.001$, respectively] (see below for explanation).

The tendency of energy expenditure (Fig. 1) and activity (Fig. 3), and to a lesser degree respiratory quotient (Fig. 2), to start at high levels and decrease over time has been repeatedly found in this paradigm, and has been ascribed to the stress of the handling and injection procedures (12–17,21) and the attenuation of these effects over time. Both the magnitude and time course of these effects are similar to those reported elsewhere (12–17,21).

DISCUSSION

The present study shows that excitatory amino acid stimulation of PVN neurons has large effects on the metabolic aspects of energy balance. Different doses of DLH increased thermogenesis and respiratory quotient, which suggests the independence of these two metabolic parameters. The increased thermogenesis reflects catabolism and negative energy balance, whereas the increased respiratory quotient indicates a shift towards the utilization of carbohydrates and the sparing of fats (3). The latter processes reflect anabolism and positive energy balance. Although it is questionable whether energy expenditure is fully synonymous with thermogenesis, there is, at the very least, an extremely close correspondence between these terms (3,6).

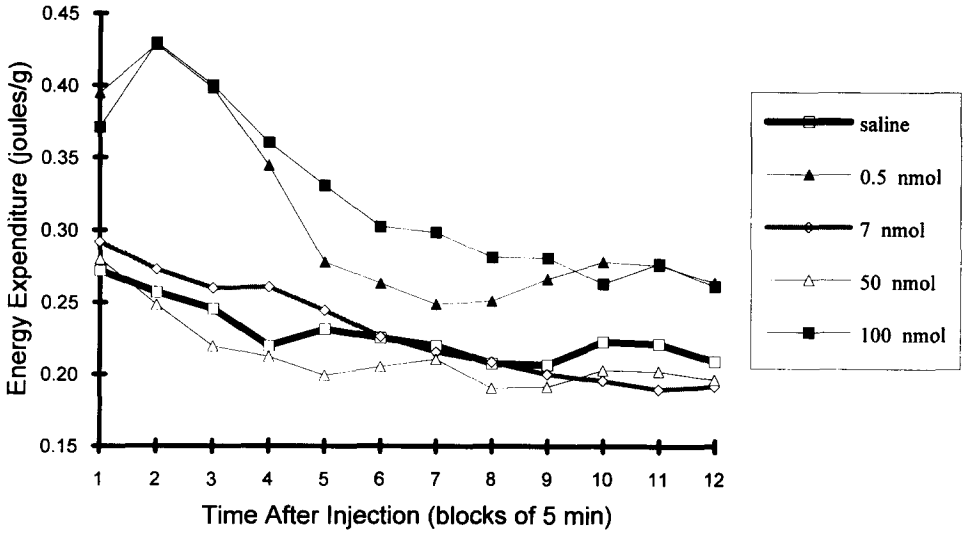

FIG. 1. Energy expenditure (J/g) over a 60-min period immediately after the injection of saline or D,L-homocysteic acid. For clarity of illustration, the data are presented in blocks of 5 min and SEM values (range 0.003–0.024) are not included.

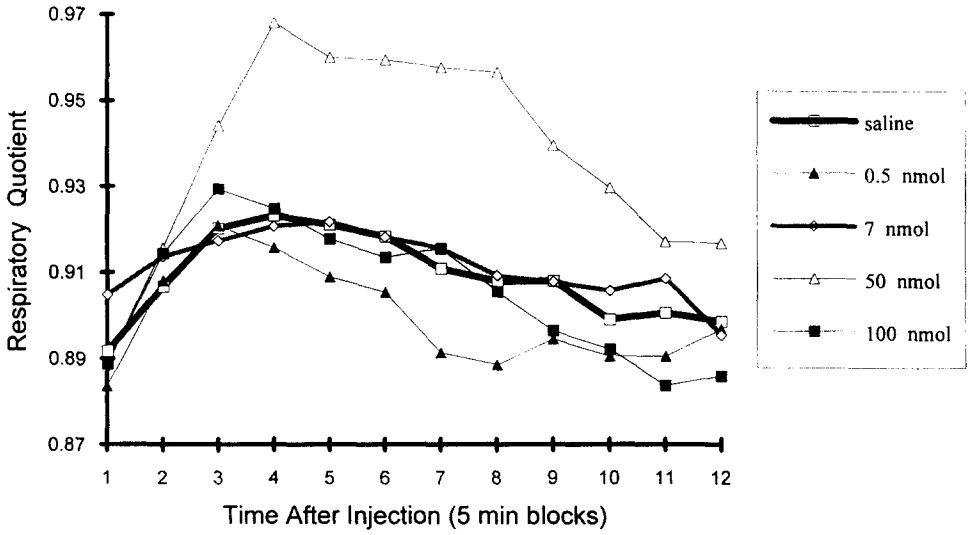

FIG. 2. Mean respiratory quotient over a 60-min period immediately after the injection of saline or D,L-homocysteic acid. For clarity of illustration, the data are presented in blocks of 5 min and SEM values (range 0.003–0.029) are not included.

The dose-response relationship of the thermogenic effect of DLH in the PVN has some parallels with DLH's shift from producing excitation at low doses to inhibition at high doses. Lipski et al. (10) report that the transition from excitation to inhibition takes place at concentrations around 5 nmol. We found strong excitation of thermogenesis well below this dose (0.5 nmol). In contrast, just above it (7 nmol), there was no overall effect on thermogenesis, but there was a within-session transition from initial thermogenic excitation to terminal thermogenic inhibition. Moreover, the thermogenic changes at all doses below 100 nmol occurred in the absence of effects on locomotor activity. This dissociation indicates primary thermogenic effects as opposed to those that are secondary to changes in locomotor activity. Thus, excitation of the neurons in the PVN increases thermogenesis, whereas inhibition of these neurons has little effect. This suggests that the inhibition of thermogenesis produced by norepinephrine and galanin injected into the PVN (16,21) is subserved by different system(s) from those affected by DLH.

In contrast to the primary thermogenic effect seen at the lowest three doses, the increased thermogenesis at the 100 nmol dose was accompanied by increased motor activity, particularly in the early part of the test session. Since this thermogenesis appears to be secondary to, or driven by, increased locomotor activity, it has little import for the role of the PVN in thermogenesis. On the other hand, these data do show a

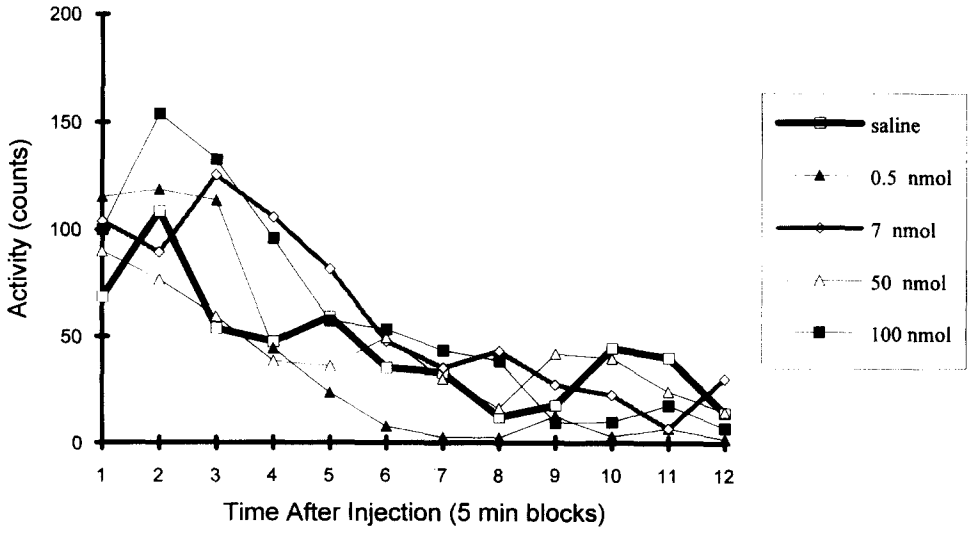

FIG. 3. Mean locomotor activity (activity counts) over a 60-min period immediately after the injection of saline or D,L-homocysteic acid. For clarity of illustration, the data are presented in blocks of 5 min and SEM values (range 0.27–42.80) are not included.

previously undocumented role for the PVN in modulating motor activity. Stimulation of motor activity has previously been reported for high doses of DLH, as well as for other excitatory amino acids, in other brain regions (13,20). However, the finding that this locomotor excitation was only seen at the highest dose raises the possibility that the motor activation does not represent a local inhibition. It could equally well represent the excitation of areas around the PVN in the periphery of the area affected by the injection.

We have previously reported that different dose of neuropeptide Y and insulin injected into the PVN produce very different, even opposite, effects (15,17). Whether these striking differentiations reflect shifts from excitation to inhibition of the same neural system(s), or the recruitment of different neural systems, remains to be determined. However, this property is not unique to the PVN. We have also shown similar functional relationships with DLH injected into the sulcal prefrontal cortex (13).

The thermogenic data presented here constitute the first direct demonstration that the PVN has an excitatory role in whole-body thermogenesis. Previous studies have focussed only on regional thermogenesis, such as the activity of interscapular brown adipose tissue (1). The present findings further support the view that the increased body weight following PVN lesions (8,19,22) may reflect decreased thermogenesis in addition to increased food intake. These joint anabolic processes would constitute a strong weight-increasing force.

The 50 nmol dose of DLH increased the respiratory quotient to a peak of around 0.97 about 20 min after the injection. The respiratory quotient is an index of energy substrate utilization (2,3,6). Under normal physiological conditions, values of around 0.90 are produced by the mixed utilization of carbohydrates, fats, and proteins. Values approaching 0.70 reflect the exclusive catabolism of fats seen, for example, in fasting. Values approaching 1.00 reflect the exclusive catabolism of carbohydrates commonly seen after a meal (2,3,6), and obtained here with the 50 nmol dose of DLH. Thus, the effect of the 50 nmol dose may be considered anabolic even though it had no effect on energy expenditure.

It is important to differentiate RQ increases that reflect increased catabolism of carbohydrates from those that may also be produced by hyperventilation. Whereas the present experiments did not concurrently monitor respiration rate, it remains very unlikely that the observed increases represent ventilatory effects. In a small animal such as the rat, hyperventilation depletes tissue reserves of CO_2 in a matter of a few minutes (6). Thus, hyperventilation is a plausible explanation of only very short-lived increases in RQ. In contrast, the RQ increases reported here persisted for at least 50 min. It therefore remains possible that hyperventilation could contribute to the early response, but it is not plausible that it could be responsible for such a sustained increase in RQ.

The increased respiratory quotient produced by the 50 nmol dose of DLH suggests that the affected population of PVN neurons normally inhibits respiratory quotient. The depolarization blockade likely caused by this high dose would disrupt this inhibition. However, this view does not readily explain the lack of effects on respiratory quotient seen at the still larger and presumably even more inhibitory dose of DLH. As with the enhanced locomotor activity seen with the very large dose, this effect may reflect the excitation of neurons in adjacent brain areas. If these adjacent neurons were also chronically inhibitory, they might well be excited by the low DLH concentrations at the periphery of the injection. In this case, the inhibition caused by the depolarization blockade in regions of high DLH concentration would be effectively cancelled by excitation in peripheral areas where the DLH concentration was low. However, at the moment, this is only speculation.

The PVN has anatomical and functional links with the sympathetic nervous system (SNS) (1,11). We have recently shown that the PVN integrates metabolic signals from the periphery with SNS-mediated neurohumoral control of energy intake, energy substrate utilization, and thermogenesis (15). In this model, the PVN is a sensor for signals, such as insulin, that indicate excessive body fat. We have shown that injections of high doses of insulin into the PVN shift whole-body metabolism toward catabolism (15). The SNS facilitates catabolism by increasing thermogenesis, inhibiting food intake, and stimulating the preferential combustion of fats (15). SNS stimulation may therefore be one of the mechanism by which the low doses of DLH increase thermogenesis.

Whereas it is not clear whether the effects on energy substrate utilization are also mediated by the SNS, the present findings are clearly relevant to the reported effects of PVN regulation on macronutrient selection (9). Given a metabolic shift toward carbohydrate utilization, it is homeostatically reasonable that the animal would show increased preference for carbohydrates.

In summary, the present data further extend the role of the PVN in modulating the metabolic aspects of energy balance. Excitation of the PVN with low doses of DLH increases thermogenesis, yet has no effects on respiratory quotient and locomotor activity. Conversely, inhibition of the PVN with higher doses of DLH increases dependence on carbohydrate as an energy substrate. At these doses, thermogenesis and locomotor activity are unaffected. Taken together, these data suggest that the excitation and inhibition of both thermogenesis and energy substrate utilization by the PVN are likely subserved by different neural systems.

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