Leptin depolarizes rat hypothalamic paraventricular nucleus neurons

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The paraventricular nucleus (PVN) is one structure that has been implicated in regulating feeding behavior. The paraventricular nucleus (PVN) is one structure that has been implicated in regulating feeding behavior. Using patch-clamp recording techniques, this study examines the direct membrane effects of leptin on neurons in a coronal PVN slice. Bath application of the physiologically active leptin fragment (amino acids 22–56) elicited dose-related depolarizations in 82% of the type I cells tested (n = 17) and 67% of the type II cells tested (n = 9). By contrast, the physiologically inactive leptin fragment (amino acids 57–92) had no discernible effect on membrane potential (n = 7). The effects of this peptide were unaffected following synaptic isolation of the cells by bath application of the sodium channel blocker tetrodotoxin (n = 5). Voltage clamp recordings in six cells demonstrated that leptin increased a nonspecific cation conductance with a reversal potential near −30 mV. These findings suggest that neurons in PVN may play an important role in the central neuronal circuitry involved in the physiological response to leptin.

METHODS

Slice preparation. Male Sprague-Dawley rats (150–250 g; Charles River) were killed by decapitation, and the brain was quickly removed from the skull and immersed in cold (1–4°C) artificial cerebrospinal fluid (aCSF). The brain was blocked, and 400-µm hypothalamic slices, including the PVN, were prepared as described previously (2). Electrophysiological techniques. Whole cell recordings were obtained using pipettes (resistance of 4–6 MΩ) filled with a solution containing (in mM) 140 K-glucuronate, 0.1 CaCl₂, 2 MgCl₂, 1.1 EGTA, 10 HEPES, 2 Na₃ATP, and adjusted at pH 7.25 with KOH. An Ag-AgCl electrode connected to the bath solution via a KCl-agar bridge served as reference. All signals were processed with an Axoclamp-2A amplifier, digitized using the CED 1401 plus interface, and stored on computer for offline analysis.

Solutions. The aCSF composition was (in mM) 124 NaCl, 2 KCl, 1.25 KPO₄, 2.0 CaCl₂, 1.3 MgSO₄, 20 NaHCO₃, and 10 glucose. Osmolarity was maintained between 285 and 300 mosM and pH between 7.3 and 7.4. Leptin fragment 22–56 (Phoenix Pharmaceuticals) was dissolved in aCSF to a stock concentration of 1 µM and then frozen until use. The leptin fragment 57–92 (Phoenix Pharmaceuticals) was first dissolved in 50–100 µl of acetonitrile, then diluted to a 1 µM stock concentration with aCSF and frozen until use. The 22–56 leptin fragment has been shown to induce significant satiety effects in the rat, whereas leptin fragment 57–92 yielded no significant alteration in feeding behavior (25).

All drugs were bath applied at the concentrations specified in the text and legends to all figures. The timing of drug application (2–8 s, resulting in a volume of 200–800 µl), is indicated by the bars in Fig. 1, which represent the time period for which the peptide in aCSF delivers fluid to the bath.
entry line. There is a delay of 30–45 s between this time and access on the peptide into the bath.

**RESULTS**

Whole cell recordings were obtained from 59 PVN neurons. These cells had resting membrane potentials negative to $-255$ mV and input resistances greater than 750 MΩ. Before leptin application, all cells were electrophysiologically classified according to previously established criteria (29). Thirty-two cells exhibited properties (delay to first spike, linear current-voltage relationship) that are characteristic of type I PVN neurons. Twenty cells exhibited properties (low-threshold Ca$^{2+}$ spike, inward rectification of current-voltage relationship at negative potentials) similar to type II cells. Seven cells could not be clearly differentiated into either type I or type II.

Effects of leptin on membrane potential. Bath application of 10 nM leptin to a slice previously unexposed to leptin depolarized 82% of the type I cells tested ($n = 17$). In addition, 67% of the type II cells tested ($n = 9$) were also depolarized by the active leptin fragment. After a minimum of 30 min, some slices were retested with a 10 nM dose of leptin ($n = 9$). Six of the cells tested in previously exposed slices did not depolarize, suggesting that there may be a slow desensitization to leptin. According to our calculations, this dose and application protocol resulted in the delivery of 2–8 pmol (200–800 µl of 10 nM solution) of leptin to the slice. Increasing the dose of leptin produced more...
pronounced depolarizations (n = 5) (Fig. 1). In some of the cells tested with the 100 nM dose, there was no recovery of the membrane potential even after 15 min (n = 2). The minimum dose required to elicit an effect on membrane potential was 0.1 nM (n = 5). The dose-response curve generated from testing doses from 0.1 to 100 nM is depicted in Fig. 2. From this curve, the EC_{50} was determined to be 29 nM. Similar depolarizations were observed in response to bath application of leptin during synaptic isolation of the cells by bath application of the sodium channel blocker tetrodotoxin (2 µM, n = 5). To rule out the possibility of nonspecific actions of this peptide on the membrane potential of PVN neurons, seven cells were tested with the physiologically inactive 57—92 leptin fragment. This fragment has previously been shown to have no significant effect on feeding behavior following intracerebroventricular application (25). In contrast to the depolarization induced by leptin-(22—56), bath application of 10 nM of leptin-(57—92) had no discernible effect on the membrane potential (Fig. 1). In three cells that did not depolarize in response to the inactive leptin fragment, reversible depolarizations were observed following application of the active leptin fragment.

Effects of leptin on membrane conductance. Current-voltage relationships were examined in six cells using slow voltage ramps (5 mV/s) under whole cell voltage clamp conditions. Under control conditions, these ramps elicited voltage-dependent currents (indicated by nonlinearity over voltage range) with a reversal potential (zero current) between −25 and −30 mV (see Fig. 3), indicating this current to be carried by a nonspecific mix of cations. Bath application of the active leptin fragment increased the amplitude of the inward current elicited by these ramps. Subtraction of control current from leptin current revealed that the difference
(i.e., leptin induced) current was linear (Fig. 3B), indicating an effect of the peptide on a voltage-independent conductance. In addition, the observation that large inward currents were induced by leptin at voltages around the resting membrane potential of the cells (−55 mV) are consistent with the depolarizing actions of this peptide observed in current clamp recordings. This difference current (Fig. 3B) exhibited a reversal potential of −28.2 ± 1.5 mV (n = 6), which is again consistent with the activation of a conductance carried by nonspecific mix of cations.

**DISCUSSION**

The results of this study demonstrate that the physiologically active leptin fragment 22–56 depolarizes neurons in the PVN. The effects of leptin likely result from the activation of a nonspecific cationic conductance as indicated by our observation that the difference current (leptin – control) in response to slow voltage ramps reverses at about −30 mV. These effects were observed in both type I and type II neurons, suggesting that the initiation of a cellular signal by this peptide may have diverse physiological consequences. The lack of effect following application of the inactive fragment suggests the response observed in response to active leptin is likely transduced by receptors that are specific for this fragment. This effect of leptin, which brings the membrane potential of PVN cells closer to the threshold for spike activation, is consistent with studies demonstrating an increase in the expression of c-fos in PVN following leptin application (5, 30, 31). The dose-related effects observed in the present study are consistent with previous work demonstrating a dose dependency of leptin effects on feeding (4, 22, 25).

The mechanisms responsible for the requisite decrease in feeding behavior following this activation of PVN neurons are unclear, but when examined in the context of previously defined effects of other substances that influence feeding behavior, a clear picture regarding the role of PVN as a satiety center begins to emerge. Substances that increase feeding behavior, such as neuropeptide Y, norepinephrine, GABA, morphine, and other opiates, inhibit neuronal firing in the PVN (21, 23, 24). In agreement with this, we report that leptin, a peptide that acts as a satiety signal and inhibits feeding, depolarizes PVN neurons, thereby increasing the likelihood they will discharge action potentials. This provides further evidence that regulating the excitability of cells within this nucleus may be a critical step in controlling feeding behavior.

In a previous study, leptin decreased input resistance and inhibited evoked excitatory postsynaptic currents in the arcuate nucleus (8). Although the doses of leptin used in the two studies, 25 nM in the arcuate nucleus versus 10 nM for a consistent depolarization in PVN, are comparable, the durations of leptin application (1–2 min vs. 2–8 s) are very different. Consequently, the neurons in the arcuate nucleus were exposed to a much larger effective dose than those in the PVN. According to our calculations, PVN neurons were exposed to between 2 and 8 pmol of leptin. These findings suggest that neurons in PVN may have a greater sensitivity to leptin than neurons in the arcuate nucleus. We would propose that PVN, although not the exclusive site of leptin actions, may be the primary central site coordinating the physiological response to leptin.

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**REFERENCES**


