Figure 5.1  Effects of 0.0 mM ATP in the patch pipette on spontaneous activity and resting membrane properties of ARC neurones in slices from rats fed *ad libitum* and fasted for 24 hours

**A:** Continuous current-clamp recording of an ARC neurone in 2.0 mM extracellular glucose, recorded in a slice from a rat fed *ad libitum*. The regular downward deflections, shown in this and subsequent figures, are membrane responses to negative rectangular-wave current pulse injection (0.2 Hz, 1.0 s, 5-20 pA) used to monitor changes in input resistance. Left, an example of a recording showing a stable baseline, and right, an example showing spontaneous inhibition.

**B:** Continuous current-clamp recording of an ARC neurone in 2.0 mM extracellular glucose, recorded in a slice from a rat fasted for 24 hours. Left, an example of a recording showing a stable baseline, and right, an example showing spontaneous inhibition.

Figure 5.2  Effects of 1.0 mM ATP in the patch pipette on spontaneous activity and resting membrane properties of ARC neurones in slices from rats fed *ad libitum* and fasted for 24 hours

**A:** Continuous current-clamp recording of an ARC neurone in 2.0 mM extracellular glucose, recorded in a slice from a rat fed *ad libitum*. Left, an example of a recording showing a stable baseline and right, an example showing spontaneous inhibition.

**B:** Continuous current-clamp recording of an ARC neurone in 2.0 mM extracellular glucose, recorded in a slice from a rat fasted for 24 hours. Left, an example of a recording showing a stable baseline, and right, an example showing spontaneous inhibition.
Figure 5.3  Effects of 2.0 mM ATP in the patch pipette on spontaneous activity and resting membrane properties of ARC neurones in slices from rats fed ad libitum and fasted for 24 hours

A: Continuous current-clamp recording of an ARC neurone in 2.0 mM extracellular glucose, recorded in a slice from a rat fed ad libitum. Left, an example of a recording showing a stable baseline and right, an example showing spontaneous inhibition.

B: Continuous current-clamp recording of an ARC neurone in 2.0 mM extracellular glucose, recorded in a slice from a rat fasted for 24 hours. Left, an example of a recording showing a stable baseline, and right, an example showing spontaneous inhibition.

Figure 5.4  Effects of 5.0 mM ATP in the patch pipette on spontaneous activity and resting membrane properties of ARC neurones in slices from rats fed ad libitum and fasted for 24 hours

A: Continuous current-clamp recording of an ARC neurone in 2.0 mM extracellular glucose, recorded in a slice from a rat fed ad libitum. Left, an example of a recording showing a stable baseline and right, an example showing spontaneous inhibition.

B: Continuous current-clamp recording of an ARC neurone in 2.0 mM extracellular glucose, recorded in a slice from a rat fasted for 24 hours. Left, an example of a recording showing a stable baseline and right, an example showing spontaneous inhibition.
Figure 5.5
Figure 5.6 Reducing
Figure 5.7
Figure 5.8
Figure 5.10 Glucose rapidly adapting neurons (GRA neurones) recorded from ARC neurons were characterized by an initial hyperpolarisation in response to an increase in extracellular glucose followed by adaptation characterized by depolarization and an increase in firing

Continuous current-clamp recording of an ARC neurone recorded in hypothalamic slices from rats fasted for 24 hours with no ATP in the pipette solution. Removal of glucose from the bathing solution induced an initial depolarization associated with an increase in firing frequency and input resistance. Subsequent increase in glucose, returning to 2.0 mM extracellular glucose, induced a large amplitude hyperpolarisation associated with decrease in firing frequency and input resistance, followed by an apparent adaptation characterised by membrane depolarisation associated with an increase in input resistance and firing frequency. The // denote approximately a two minute break within the trace. This adaptive response to increased glucose was repeatable upon re-exposure of the slice to 0.0 mM followed by 2.0 mM extracellular glucose. The // denote approximately two minutes breaks within the trace.
Figure 5.11
Figure 5.13
Figure 5.14
Figure 5.15  Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible inhibition in some ARC neurones (GE neurones) recorded from rats fed ad libitum with 2.0 mM ATP in the recording pipette

A: Continuous current-clamp recording from an ARC neurone in a slice from a rat fed ad libitum with 2.0 mM ATP in the pipette. Removal of glucose from the bathing solution induced a decrease in firing frequency and an associated reduction in input resistance (the latter indicated by a fall in amplitude of the membrane responses to current injection). This effect of 0.0 mM glucose was reversed by returning to a 2.0 mM extracellular glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A) for control (2.0 mM glucose) and glucose-free aCSF. The records show superimposed samples of a continuous whole-cell current-clamp recording showing membrane potential responses to a series of depolarising and hyperpolarising rectangular-wave current injections of constant increment.
Figure 5.16
Figure 5.17 Reducing extracellular glucose from 2.0 to 0.0 mM induced excitation in some ARC neurones (GI neurones) recorded from rats fed *ad libitum* with 2.0 mM ATP in the recording pipette

A: Continuous current-clamp recording of an ARC neurone recorded in slices from rats fed *ad libitum* rats, utilising a pipette solution with intracellular ATP concentration of 2.0 mM. The regular downward deflections are membrane responses to negative current pulse injection (0.2 Hz, 1.0 s, 5-20 pA). Removal of glucose from the bathing solution induced depolarisation, an increase in firing frequency and an associated increase in input resistance. This effect of 0.0 mM glucose reversed was irreversible after wash in 2.0 mM extracellular glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A) for control (2.0 mM glucose) and glucose-free aCSF. The records show superimposed samples of a continuous whole-cell current-clamp recording showing membrane potential responses to a series of depolarising and hyperpolarising rectangular-wave current injections of constant increment.
Figure 5.18 Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible inhibition in some ARC neurones (GE neurones) recorded from rats fed *ad libitum* with 5.0 mM ATP in the recording pipette

A: Continuous current-clamp recording from an ARC neurone in a slice from a rat fed *ad libitum* with 5.0 mM ATP in the pipette. Removal of glucose from the bathing solution induced membrane hyperpolarisation, a decrease in firing frequency and an associated reduction in input resistance (the latter indicated by a fall in amplitude of the membrane responses to current injection). This effect of 0.0 mM glucose was reversed by returning to a 2.0 mM extracellular glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A) for control (2.0 mM glucose) and glucose-free aCSF. The records show superimposed samples of a continuous whole-cell current-clamp recording showing membrane potential responses to a series of depolarising and hyperpolarising rectangular-wave current injections of constant increment.
Figure 5.19 Reducing extracellular glucose from 2.0 to 0.0 mM induced an inhibition in some ARC neurones (GE neurones) recorded from 24-hour fasted rats with 5.0 mM ATP in the recording pipette

A: Continuous current-clamp recording from an ARC neurone in a slice from a fasted rat with 5.0 mM ATP in the pipette. Removal of glucose from the bathing solution induced membrane hyperpolarisation, a decrease in firing frequency and an associated reduction in input resistance. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A) for control (2.0 mM glucose) and glucose-free aCSF. The records show superimposed samples of a continuous whole-cell current-clamp recording showing membrane potential responses to a series of depolarising and hyperpolarising rectangular-wave current injections of constant increment.
**Figure 5.20** Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible excitation in some ARC neurones (GI neurones) recorded from rats fed *ad libitum* with 5.0 mM ATP in the recording pipette

**A:** Continuous current-clamp recording of an ARC neurone recorded in slices from rats fed *ad libitum* rats, utilising a pipette solution with intracellular ATP concentration of 5.0 mM. The regular downward deflections are membrane responses to negative current pulse injection (0.2 Hz, 1.0 s, 5-20 pA). Removal of glucose from the bathing solution induced depolarisation, an increase in firing frequency and an associated decrease in input resistance. This effect of 0.0 mM glucose was reversed after wash in 2.0 mM extracellular glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

**B:** I-V relationships obtained from the neurone shown in (A): control (2.0 mM glucose) and in the presence of glucose-free aCSF.

**C:** A plot of data shown in B (current shown on the x-axis and the resulting membrane voltage responses on the y-axis). The current-voltage (I-V) relationships in the presence of 2.0 mM (■) and 0.0 mM (□) were shown, giving a reversal potential around -44 mV, suggesting activation of one or more non-selective cation conductances in 0.0 mM glucose. In addition, the I-V plots for 0.0 mM (□) and reapplication of 2.0 mM glucose (▲) revealed a reversal potential at the same potential around -44 mV, further suggesting activation of non-selective cation conductances underlies these effects of changes in extracellular glucose.
Figure 5.21 Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible excitation in some ARC neurones (GI neurones) recorded from rats fed ad libitum with 5.0 mM ATP in the recording pipette

A: Continuous current-clamp recording of an ARC neurone recorded in slices from rats fed ad libitum rats, utilising a pipette solution with intracellular ATP concentration of 5.0 mM. The regular downward deflections are membrane responses to negative current pulse injection (0.2 Hz, 1.0 s, 5-20 pA). Removal of glucose from the bathing solution induced depolarisation, an increase in firing frequency and an associated decrease in input resistance. This effect of 0.0 mM glucose was reversed after wash in 2.0 mM extracellular glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A): control (2.0 mM glucose), in the presence of glucose-free aCSF and following wash in 2.0 mM glucose.
Figure 5.22
Figure 5.23 Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible inhibition in some ARC neurones (GE neurones) recorded from 24-hour fasted rats with 10.0 mM ATP in the recording pipette

A: Continuous current-clamp recording from an ARC neurone in a slice from a fasted rat with 10.0 mM ATP in the pipette. Removal of glucose from the bathing solution induced membrane hyperpolarisation, a decrease in firing frequency and an associated reduction in input resistance. These effects were reversible on wash with 2.0 mM glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A) for control (2.0 mM glucose), glucose-free aCSF and wash in 2.0 mM glucose-containing aCSF. The records show superimposed samples of a continuous whole-cell current-clamp recording showing membrane potential responses to a series of depolarising and hyperpolarising rectangular-wave current injections of constant increment.
Figure 5.24
Figure 5.25
Figure 5.26 Summary of the responses detected in ARC neurones, in hypothalamic slices prepared from rats fed *ad libitum*, following a reduction in extracellular glucose from 2.0 to 0.0 mM with a range of intracellular ATP concentrations

Pie charts showing the range and proportion of responses of ARC neurones to a reduction in extracellular glucose (2.0 to 0.0 mM), with a range of intracellular ATP concentrations, recorded in hypothalamic slice preparations from rats fed *ad libitum*. Intracellular ATP concentrations used were (A) 0.0 mM, (B) 1.0 mM, (C) 2.0 mM, (D) 5.0 mM and (E) 10.0 mM. Four types of response to a reduction in extracellular glucose were detected in ARC neurones: inhibition (GE, blue), excitation (GI, grey), rapidly adapting (GRA, purple) and no response (black). A total of 63 ARC neurones were recorded in this study from rats fed *ad libitum* (11 neurones in 0.0 mM intracellular ATP; 14 neurones in 1.0 mM intracellular ATP; 9 neurones in 2.0 mM intracellular ATP; 18 neurones in 5.0 mM intracellular ATP and 11 neurones in 10.0 mM intracellular ATP.

Figure 5.27 Summary of the responses detected in ARC neurones, in hypothalamic slices prepared from 24-hour fasted rats, following a reduction in extracellular glucose from 2.0 to 0.0 mM with a range of intracellular ATP concentrations

Pie charts showing the range and proportion of responses of ARC neurones to a reduction in extracellular glucose (2.0 to 0.0 mM), with a range of intracellular ATP concentrations, recorded in hypothalamic slice preparations from 24-hour fasted rats. Intracellular ATP concentrations used were (A) 0.0 mM, (B) 1.0 mM, (C) 2.0 mM, (D) 5.0 mM and (E) 10.0 mM. Four types of response to a reduction in extracellular glucose were detected in ARC neurones: inhibition (GE, blue), excitation (GI, grey), rapidly adapting (GRA, purple) and no response (black). A total of 55 ARC neurones were recorded in this study from 24-hour fasted rats (9 neurones in 0.0 mM intracellular ATP; 8 neurones in 1.0 mM intracellular ATP; 10 neurones in 2.0 mM intracellular ATP; 11 neurones in 5.0 mM intracellular ATP and 17 neurones in 10.0 mM intracellular ATP.
Figure 5.28 Tolbutamide reverses the inhibitory effects of glucose-free bathing medium in ARC GE neurones recorded from hypothalamic slices prepared from rats fed *ad libitum*

A: Continuous current-clamp recording of an ARC neurone recorded in a hypothalamic slice prepared from a rat fed *ad libitum*, utilising a pipette solution from which intracellular ATP was omitted. This neurone responded to glucose-free bathing medium with membrane hyperpolarisation, a decrease in firing frequency and an associated reduction in input resistance. Subsequently, in the presence of TTX (500 nM), to eliminate indirect circuit-dependent effects, bath application of tolbutamide (200 μM) reversed the effects of glucose-free aCSF inducing membrane depolarisation associated with an increase in neuronal input resistance. The // denote approximately two minutes breaks within the trace for generating I-V relationships.

B: Superimposed traces of membrane responses of an ARC neurone to a range of hyperpolarising and depolarising rectangular-wave current steps of constant increment. The records were obtained from the same neurone as shown in (A): in control, in glucose-free aCSF, in glucose-free aCSF + TTX and subsequently in the presence of tolbutamide.

C: Superimposed currents obtained in response to voltage-clamp ramps in glucose-free bathing medium and TTX (black trace) and subsequently in the presence of tolbutamide (red trace). The currents were obtained from ramp protocols that drove the holding potential from -120.0 mV to -40.0 mV, at a rate of 10.0 mV per second. The point of intersection of the current responses indicates the reversal potential of the tolbutamide-induced current (approximately -83 mV), close to the predicted reversal potential for potassium under our recording conditions. These data indicate that tolbutamide-sensitive $K_{\text{ATP}}$ channels underpin responses to glucose-free bathing medium.
Figure 5.3

A. Fed *ad libitum*

B. 24-hour fasted

Figure 5.4

A. Fed *ad libitum*

B. 24-hour fasted
Figure 5.5  Effects of 10.0 mM ATP in the patch pipette on spontaneous activity and resting membrane properties of ARC neurones in slices from rats fasted for 24 hours

Continuous current-clamp recording of an ARC neurone in 2.0 mM extracellular glucose, recorded in a slice from a rat fasted for 24 hours. Top, an example of a recording showing a stable baseline and bottom, an example showing spontaneous excitation.
Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible inhibition in some ARC neurones (GE neurones) recorded from rats fed *ad libitum* with 0.0 mM ATP in the recording pipette.

**A:** Continuous current-clamp recording from an ARC neurone in a slice from a rat fed *ad libitum* with 0.0 mM ATP in the pipette. Removal of glucose from the bathing solution induced membrane hyperpolarisation, a decrease in firing frequency and an associated reduction in input resistance (the latter indicated by a fall in amplitude of the membrane responses to current injection). This effect of 0.0 mM glucose was reversed by returning to a 2.0 mM extracellular glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

**B:** I-V relationships obtained from the neurone shown in (A) for control (2.0 mM glucose) and glucose-free aCSF. The records show superimposed samples of a continuous whole-cell current-clamp recording showing membrane potential responses to a series of depolarising and hyperpolarising rectangular-wave current injections of constant increment.
Figure 5.7

A: Continuous current-clamp recording from an ARC neurone in a slice from a fasted rat with no ATP in the pipette. Removal of glucose from the bathing solution induced membrane hyperpolarisation, a decrease in firing frequency and an associated reduction in input resistance (the latter indicated by a fall in amplitude of the membrane responses to current injection). This effect of 0.0 mM glucose was reversed by returning to a 2.0 mM extracellular glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A) for control (2.0 mM glucose) and glucose-free aCSF. The records show superimposed samples of a continuous whole-cell current-clamp recording showing membrane potential responses to a series of depolarising and hyperpolarising rectangular-wave current injections of constant increment.

Figure 5.7 Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible inhibition in some ARC neurones (GE neurones) recorded from 24-hour fasted rats with 0.0 mM ATP in the recording pipette
Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible excitation in some ARC neurones (GI neurones) recorded from rats fed *ad libitum* with 0.0 mM ATP in the recording pipette.

Continuous current-clamp recording of an ARC neurone recorded in slices from rats fed *ad libitum* rats, utilising a pipette solution from which intracellular ATP was omitted. The regular downward deflections are membrane responses to negative current pulse injection (0.2 Hz, 1.0 s, 5-20 pA). Removal of glucose from the bathing solution induced depolarisation, an increase in firing frequency and an associated reduction in input resistance. This effect of 0.0 mM glucose was reversed by returning to a 2.0 mM extracellular glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.
Figure 5.9

A

Reducing extracellular, glucose from 2.0 to 0.0 mM, induced excitation in some ARC neurones (GI neurones) recorded from 24-hour fasted rats with 0.0 mM ATP in the recording pipette

A: Continuous current-clamp recording of an ARC neurone recorded in slices from 24-hour fasted rats, utilising a pipette solution with intracellular ATP concentration of 0.0 mM. Removal of glucose from the bathing solution induced depolarisation, an increase in firing frequency and an associated decrease in input resistance. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A): control (2.0 mM glucose) and in the presence of glucose-free aCSF.
Figure 5.10

Glucose 2.0 mM

-42.0 mV

Glucose 0.0 mM

-39.0 mV

Glucose 2.0 mM

-43.0 mV

Glucose 0.0 mM

-39.0 mV

Glucose 2.0 mM
Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible inhibition in some ARC neurones (GE neurones) recorded from rats fed *ad libitum* with 1.0 mM ATP in the recording pipette.

**A:** Continuous current-clamp recording from an ARC neurone in a slice from a rat fed *ad libitum* with 1.0 mM ATP in the pipette. Removal of glucose from the bathing solution induced membrane hyperpolarisation, a decrease in firing frequency and an associated reduction in input resistance (the latter indicated by a fall in amplitude of the membrane responses to current injection). This effect of 0.0 mM glucose was reversed by returning to a 2.0 mM extracellular glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

**B:** I-V relationships obtained from the neurone shown in (A) for control (2.0 mM glucose) and glucose-free aCSF. The records show superimposed samples of a continuous whole-cell current-clamp recording showing membrane potential responses to a series of depolarising and hyperpolarising rectangular-wave current injections of constant increment.
Figure 5.12

Reducing extracellular glucose from 2.0 to 0.0 mM induced an inhibition in some ARC neurones (GE neurones) recorded from 24-hour fasted rats with 1.0 mM ATP in the recording pipette

A: Continuous current-clamp recording from an ARC neurone in a slice from a fasted rat with 1.0 mM ATP in the pipette. Removal of glucose from the bathing solution induced membrane hyperpolarisation, a decrease in firing frequency and an associated reduction in input resistance. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A) for control (2.0 mM glucose) and glucose-free aCSF. The records show superimposed samples of a continuous whole-cell current-clamp recording showing membrane potential responses to a series of depolarising and hyperpolarising rectangular-wave current injections of constant increment.
Figure 5.13 Reducing extracellular glucose from 2.0 to 0.0 mM induced excitation in some ARC neurones (GI neurones) recorded from 24-hour fasted rats with 1.0 mM ATP in the recording pipette.

Continuous current-clamp recording of an ARC neurone recorded in slices from 24- hour fasted rats, utilising a pipette solution with intracellular ATP concentration of 1.0 mM. Removal of glucose from the bathing solution induced depolarisation, an increase in firing frequency and an associated decrease in input resistance, an effect was reversible on returning to a 2.0 mM glucose-containing aCSF. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.
Glucose rapidly adapting neurons (GRA neurones) recorded from ARC neurons were characterized by an initial hyperpolarisation in response to an increase in extracellular glucose followed by adaptation characterized by depolarization and an increase in firing.

Continuous current-clamp recording of an ARC neurone recorded in hypothalamic slices from rats fed *ad libitum*, with 1.0 mM ATP in the pipettes solution. Removal of glucose from the bathing solution induced hyperpolarisation associated with a decrease in firing frequency and input resistance. Subsequent increase in glucose, returning to 2.0 mM extracellular glucose, induced a large amplitude hyperpolarisation associated with decrease in firing frequency and input resistance, followed by an apparent adaptation characterised by membrane depolarisation associated with an increase in input resistance. The // denote approximately a two minute break within the trace.
Figure 5.15

A

-42.0 mV

Glucose 2.0 mM

Glucose 0.0 mM

-41.0 mV

Glucose 2.0 mM

B

-43.0 mV

Glucose 2.0 mM

-40.0 mV

Glucose 0.0 mM

40 mV

600 ms

4 mins
Reducing extracellular glucose from 2.0 to 0.0 mM induced a reversible inhibition in some ARC neurones (GE neurones) recorded from 24-hour fasted rats with 2.0 mM ATP in the recording pipette.

Continuous current-clamp recording from an ARC neurone in a slice from a fasted rat with 2.0 mM ATP in the pipette. Removal of glucose from the bathing solution induced membrane hyperpolarisation, a decrease in firing frequency and an associated reduction in input resistance, an effect was reversible upon restoring extracellular glucose to 2.0 mM. These effects of 0.0 mM glucose-containing aCSF were subsequently repeatable. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.
Figure 5.17

A

Glucose 2.0 mM
-52.0 mV

Glucose 0.0 mM

5 mins

40 mV

B

Glucose 2.0 mM
-53.0 mV

Glucose 0.0 mM

600 ms

40 mV
Figure 5.18

A

Glucose 2 mM

-45.0 mV

Glucose 0 mM

-59.0 mV

4 mins

B

Glucose 2 mM (start)

-47.0 mV

-48.0 mV

Glucose 2 mM

-58.0 mV

Glucose 0 mM

-48.0 mV

600 ms
Figure 5.19

A

Glucose 2.0 mM

-43.0 mV

Glucose 0.0 mM

-47.0 mV

Glucose 2.0 mM

B

-44.0 mV

Glucose 2.0 mM (start)

-46.0 mV

Glucose 0.0 mM

-44.0 mV

Glucose 2.0 mM

600 ms

30 mV

3 mins

40 mV
Figure 5.20

A

-55.0 mV

Glucose 2.0 mM

Glucose 0.0 mM

-47.0 mV

B

-54.0 mV

Glucose 2.0 mM (start)

Glucose 0.0 mM

-47.0 mV

Glucose 2.0 mM

C

Current (pA)

-100 -50 0 50 100

-160 -140 -120 -100 -80 -60 -40 -20 0 20

-90 -80 -70 -60 -50

-40 -30 -20 -10 0 10 20

-30 -20 -10 0 10 20

-20 -10 0 10 20

-10 0 10 20

-100 0 100

2.0 mM (start)

0.0 mM

2.0 mM

Graph showing the relationship between membrane potential and current for different glucose concentrations.
Figure 5.21

A

Glucose 2.0 mM

-43.0 mV

Glucose 0.0 mM

-41.0 mV

B
Figure 5.22 Reducing extracellular, glucose from 2.0 to 0.0 mM, induced excitation in some ARC neurones (GI neurones) recorded from 24-hour fasted rats with 5.0 mM ATP in the recording pipette.

A: Continuous current-clamp recording of an ARC neurone recorded in slices from 24-hour fasted rats, utilising a pipette solution with intracellular ATP concentration of 5.0 mM. Removal of glucose from the bathing solution induced depolarisation, an increase in firing frequency and an associated increase in input resistance. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A): control (2.0 mM glucose) and in the presence of 0.2 mM glucose-containing aCSF.
Figure 5.23

A

B
Figure 5.24 Reducing extracellular glucose from 2.0 to 0.0 mM induced excitation in some ARC neurones (GI neurones) recorded from rats fed ad libitum with 10.0 mM ATP in the recording pipette.

A: Continuous current-clamp recording of an ARC neurone recorded in slices from rats fed ad libitum, utilising a pipette solution with intracellular ATP concentration of 10.0 mM. Removal of glucose from the bathing solution induced depolarisation, an increase in firing frequency and an associated decrease in input resistance. The // denote approximately two minutes breaks within the trace where the I-V relationship was generated.

B: I-V relationships obtained from the neurone shown in (A): control (2.0 mM glucose) and in the presence of glucose-free aCSF.
Figure 5.25  Effects of intracellular ATP on the time-to-peak of ARC GE and GI neurones in response to a decrease in extracellular glucose concentration, in slices prepared from rats fed *ad libitum* and 24-hour fasted rats

Bar-chart comparing the time to peak response, following a decrease in extracellular glucose concentration of ARC GE and GI neurones in slices prepared from fed (black) and fasted rats (grey) with different concentrations of ATP in the recording solution. (A) A total of 35 ARC GE-neurones were recorded in hypothalamic slices from rats fed *ad libitum* (0.0 mM ATP, n = 8; 1.0 mM ATP, n = 9; 2.0 mM ATP, n = 7; 5.0 mM ATP, n = 10; 10.0 mM ATP, n = 1). A total of 23 ARC GE-neurones were recorded in hypothalamic slices from rats fasted rats (0.0 mM ATP, n = 3; 1.0 mM ATP, n = 6; 2.0 mM ATP, n = 4; 5.0 mM ATP, n = 3; 10.0 mM ATP, n = 7). (B) A total of 14 ARC GI-neurones were recorded in hypothalamic slices from rats fed *ad libitum* (0.0 mM ATP, n = 2; 1.0 mM ATP, n = 0; 2.0 mM ATP, n = 2; 5.0 mM ATP, n = 2; 10.0 mM ATP, n = 0). A total of 6 ARC GI neurones were recorded in hypothalamic slices from rats fasted rats (0.0 mM ATP, n = 2; 1.0 mM ATP, n = 2; 2.0 mM ATP, n = 0; 5.0 mM ATP, n = 2; 10.0 mM ATP, n = 0).
Figure 5.26

A. ATP 0.0 mM

B. ATP 1.0 mM

C. ATP 2.0 mM

D. ATP 5.0 mM

E. ATP 10.0 mM

Figure 5.27

A. ATP 0.0 mM

B. ATP 1.0 mM

C. ATP 2.0 mM

D. ATP 5.0 mM

E. ATP 10.0 mM

Legend:
- Blue: Inhibition
- Gray: Excitation
- Pink: Rapidly adapting
- Black: No response
Figure 5.28

A

Glucose 0.0 mM

-42.0 mV

5 mins

30 mV

Glucose 0.0 mM + 500 nM TTX

-51.0 mV

/\109

Glucose 0.0 mM + 500 nM TTX + 200 μM Tolbutamide

B

Glucose 2.0 mM

-41.0 mV

Glucose 0.0 mM

-50.0 mV

40 mV

600 ms

-51.0 mV

Membrane Potential (mV)

C

Glucose 0.0 mM + 500 nM TTX

-39.0 mV

Glucose 0.0 mM + 500 nM TTX + 200 μM Tolbutamide

Membrane Potential (mV)

-130 -120 -110 -100 -90 -80 -70 -60 -50 -40 -30

Current (pA)

-140 -120 -100 -80 -60 -40 -20 0

-130 -120 -110 -100 -90 -80 -70 -60 -50 -40 -30

-120 -100 -80 -60 -40 -20 0

-140 -120 -100 -80 -60 -40 -20 0

-130 -120 -110 -100 -90 -80 -70 -60 -50 -40 -30

-140 -120 -100 -80 -60 -40 -20 0