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Drug receptors and the inhibition of nerve cells

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Introduction

It would be trite indeed to say much to this audience of the contributions to pharmacology made by Sir John Gaddum. His quantitative approaches to the study of drug antagonism have found their way into most textbooks of pharmacology. I dare say that, if asked to define that aspect of the subject which is uniquely 'pharmacology'—a task of increasing difficulty in these interdisciplinary days—many Society members would think first of those quantitative methods for studying drug-receptor interactions spawned by A.J. Clark, tested with antagonists by John Gaddum, and much popularized by Heinz Schild (Clark, 1933; Gaddum, 1937; 1957; Schild, 1949). I thank the Trustees for providing me with the opportunity to add my own small tribute to the memory of John Gaddum's work; this is particularly so because—as you will see—my own research has been much influenced by his contributions.

John Gaddum worked mostly with peripheral tissues. This was convenient because the tissues were readily accessible, easy to maintain *in vitro*, and in those days devices could be made to measure the appropriate response, such as contraction, relaxation or secretion; furthermore, it was generally not important to distinguish the drug effects on the individual cells within the syncytium. The first efforts to classify receptors on nerves were also made at their terminations in the periphery, following on from the well-known observations of Lindor Brown and John Gillespie (1957). That field, the study of autonomic presynaptic receptors, has matured and has led to novel therapies. But in the ganglia of the autonomic nervous system and in the central nervous system, the individual nerve cell is the functional unit, and information about drug receptors on nerve cells can best be obtained by recording from single cells. Conversely, the demonstration and characterization of drug receptors on nerve cells can itself be used as a way of classifying the cells, particularly when taken in concert with other information regarding the ion channels expressed, the transmitters synthesized and the targets to which the cells project.

Inhibition of nerve cells

If we restrict our thoughts to those relatively immediate effects on nerve cells which control their function, then the most important measure is the frequency of discharge of action potentials, and drugs either increase or decrease this. Few nerve cells in the body are silent for long; many are endowed with ion channels which open or close at different rates when the membrane potential changes and thus generate their own activity, and others are constantly bombarded with excitatory synaptic inputs. Our early insight into inhibition in the mammalian nervous system came from the work of Sir Charles Sherrington (1932) and his one-time student Jack Eccles (1964). Such inhibition causes a reduction in the excitability of motoneurones supplying opposing muscle groups when the primary (agonistic) muscles contract; the inhibition is fast, appropriate to the control of neurones that encode their information in frequencies even greater than 100 Hz. It is brought about by the opening of membrane chloride channels; chloride enters the cell tending to hyperpolarize, and the dissipation of contemporaneous excitatory synaptic currents through the open chloride channels can also contribute to the inhibition. In these cases, at least in the vertebrate nervous system, the chloride ion channels are oligomeric proteins; one or more of the subunits serves as transmitter receptor—γ-aminobutyric acid or glycine. *Drugs* can act at these receptors or at other sites on the oligomeric complex (see Barnard *et al.*, 1987).

Another way to increase the negativity inside the neurone and thus inhibit firing, is to open channels that allow potassium ions to diffuse from the cell down their concentration gradient. The mechanism was first invoked to explain the inhibitory action of acetylcholine on the heart (Burgen & Terroux, 1953; del Castillo & Katz, 1955), and was later shown to be responsible for synaptic inhibition in a number of molluscan and amphibian neurones (Tauc & Gerschenfeld, 1962; Hartzell *et al.*, 1977). In the remainder of my lecture I will review experiments which test the general hypothesis that many drugs act at their receptors on mammalian nerve cells to bring about an inhibition in cell firing, and that they do so by opening potassium channels in the cell membrane.

Characterization of receptors

The experimental work that I shall describe has been carried out on small pieces of nervous tissue maintained *in vitro*, submerged in a warm, flowing physiological solution of appropriate composition. The tip of a glass microelectrode is positioned in the cell interior so that the membrane potential can be measured. We can take advantage of having the tissue *in vitro* to use Gaddum's methods: both the agonist and the antagonist are applied for sufficiently long periods of time that their effects reach a steady-state, because the solution that flows over and through the tissue is simply changed to one that contains the drug. Figure 1 shows an experiment in which the opioid agonist [Met^5]enkephalin was applied to a noradrenaline-containing cell, in the rat locus coeruleus. A low concentration reduces the frequency of discharge, and a higher concentration stops the firing completely and hyperpolarizes the membrane.

These kinds of results can be plotted in the usual way. The results from a similar experiments carried out on other nerve cells are shown in Figure 2. In Figure 2a the neurone was from the guinea-pig submucous plexus. The agonist is again [Met^5]enkephalin and the first antagonist was naloxone; the naloxone was then washed from the tissue and the experiment was repeated using a N-bisallyl substituted enkephalin analogue from ICI (Figure 2b). Both antagonists caused a parallel shift in the dose-response curve. It turns out that the naloxone K_D was about 20 nM in this cell, which differs by a factor of ten from that on the locus coeruleus cell (Figure 1). On the other hand, the ICI compound can be used to distinguish between the receptors on the two neurones. In Figure 2c are presented

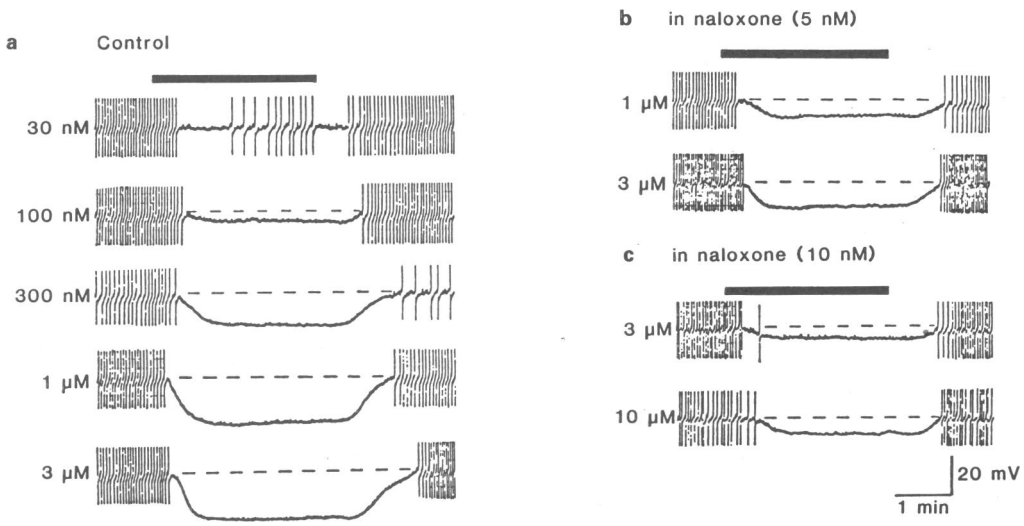

Figure 1 [Met^5]enkephalin hyperpolarizes a rat locus coeruleus neurone, and this is antagonized by naloxone. The records show the membrane potential of a spontaneously firing neurone. Upward deflections are action potentials which are not fully resolved because of the limited frequency response of the pen recorder (full amplitude was 70 mV); downward deflections are after-hyperpolarizations following the action potentials. (a) When the superfusing solution was changed to one that contained [Met^5]enkephalin (period indicated by the bar above the top trace) the firing slowed and the membrane was hyperpolarized. Higher concentrations of [Met^5]enkephalin (indicated to left of each trace) caused larger hyperpolarizations. (b & c) In naloxone, higher concentrations of [Met^5]enkephalin were required to hyperpolarize the cell. Only nine agonist applications are shown in this figure; it is often possible to apply more combinations of agonist and antagonist, but this is limited by the time for which the intracellular recording can be maintained from a single cell. Reproduced with permission from Williams & North (1984).

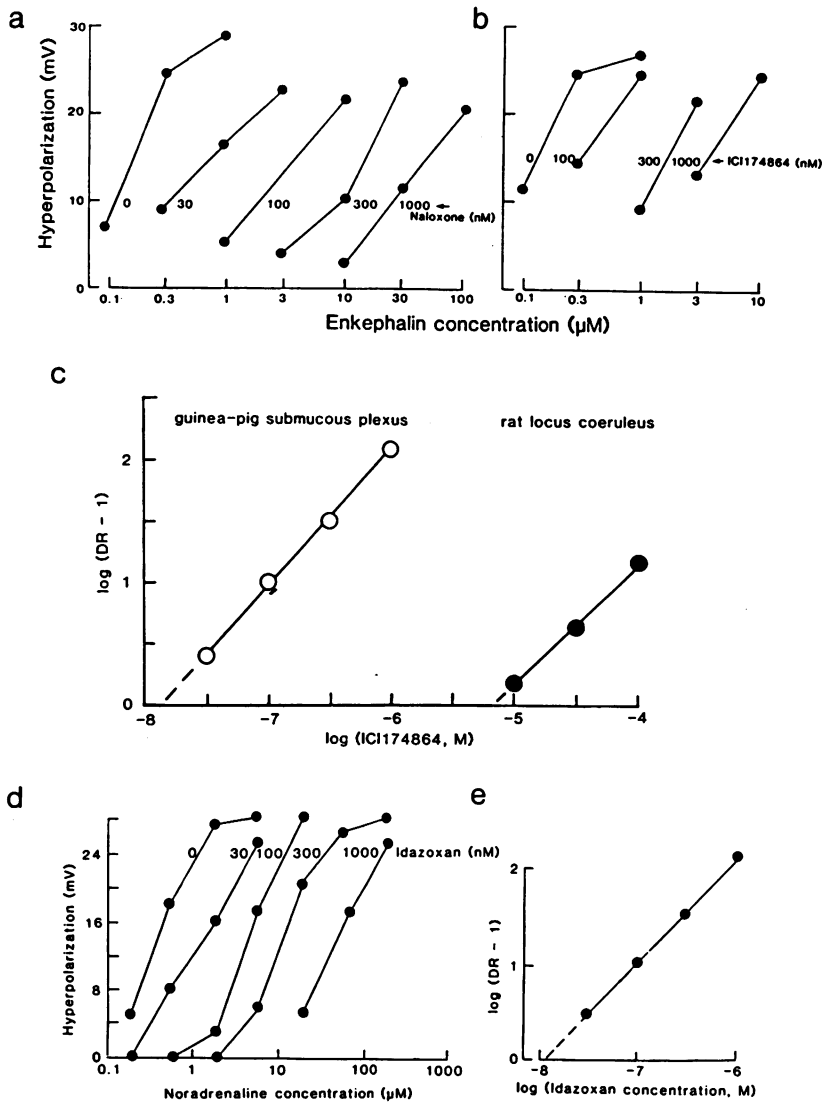

Figure 2 Examples of receptor classification on single neurones. Concentration-response curves from experiments such as those illustrated in Figure 1. (a & b), [Met^5]enkephalin hyperpolarization of a guinea-pig submucous plexus neurone: this is antagonized by naloxone (a) and by ICI174864 (*N*,*N*-bisallyl-Tyr-Aib-Aib-Phe-Leu-OH, Aib = aminoisobutyrate) (b). (c) Schild plots for the antagonism by ICI174864 of the hyperpolarizing action of [Met^5]enkephalin in a guinea-pig submucous plexus neurone (○) and a rat locus coeruleus neurone (●). The estimates of the K_D differ by almost three orders of magnitude, indicating that locus coeruleus neurones express μ-receptors and submucous plexus neurones have δ-receptors. (d) Noradrenaline hyperpolarization of a guinea-pig submucous plexus neurone. (e) Schild plot of the results in (d) indicate that the noradrenaline acts at α_2-adrenoceptors (idazoxan K_D about 10 nM). Reproduced, with permission, from Mihara & North (1986) (a & b), North (1986a) (c) and Surprenant (1986) (d & e).

the results of experiments on one neurone from each tissue; in both cases the agonist was [Met^5]enkephalin, the effect measured was hyperpolarization, and the antagonist was ICI174864. Gaddum would have concluded that these receptors are not the same: the one on the locus coeruleus is a μ-receptor, and on the submucous plexus neurone, a δ-receptor. Experiments with selective agonists such

as DAGOL (Tyr-D-Ala-Gly-MePhe-Gly-ol) and DPDPE (Tyr-D-Pen-Gly-Phe-D-Pen) that were developed later gave complementary results.

Let me provide one further example of this kind of experiment, because the receptor characterization is fundamental to much that follows. The same neurones clearly have more than one receptor that brings about hyperpolarization. Figure 2d plots the hyperpolarization caused by noradrenaline in another neurone, also from the guinea-pig submucous plexus; the antagonist was idazoxan. From the Schild plot (Figure 2e) one can obtain a fairly precise estimate of the K_D for idazoxan and we conclude that this is an α_2-adrenoceptor. Thus, submucous plexus neurones have both δ-opioid and α_2-adrenoceptors, activation of which hyperpolarizes the cell.

By such experiments we have found that not only dopamine, opioids, and noradrenaline but agonists at a number of different drug receptors result in membrane hyperpolarization and inhibition of firing on various sets of neurones (North *et al.*, 1987). Some examples of the tissues studied and the results obtained are shown in Table 1. All these neurones were acutely dissected from adult animals; under these conditions they all express at least two receptors from this *family*. We have determined antagonist dissociation equilibrium constants wherever possible, although in some cases satisfactory antagonists remain unavailable.

Potassium channels are opened

I wish to turn next to the reason why the cells hyperpolarize in the face of all these different receptor agonists. The simplest way to study this is to measure the membrane currents directly by the voltage-clamp method. Figure 3a shows an experimental recording of membrane current from a rat locus coeruleus neurone; the membrane potential is the independent variable. The control current/voltage relation (labelled 2.5) was made by slowly changing the membrane potential and recording the resulting membrane current. The fact that the line is not straight indicates that this cell membrane does not obey Ohm's law—in fact the conductance of the cell increases (or the resistance falls, or ion channels open) as the membrane is hyperpolarized. [Met^5]enkephalin causes an outward current at the resting potential, no current at about -120 mV, and an inward current at more strongly hyperpolarized potentials (outward currents are assigned positive values and depicted in the upward direction). The potential at which enkephalin causes no current, the reversal potential, is -120 mV if the extracellular potassium concentration is similar to that normally found in mammalian brain—about 2.5 mM. This reversal potential changes according to the logarithm of the extracellular potassium concentration (traces labelled 6.5), and this indicates that the action of the agonist is to open ion channels in the membrane which are selectively permeable to potassium (Williams *et al.*, 1982; 1988a). In the normal operating range of the cell this current is outward, leading to hyperpolarization.

A concentration of 100 nM enkephalin typically causes an outward current at -60 mV of 100 pA; during a 2 min application such as those illustrated, this would correspond to the loss of about 100 fmol of potassium. For a locus coeruleus neurone of about 10 μm radius, this represents about one-sixth of its potassium content. Bear in mind, however, that in order to stop the cell from firing completely, much smaller outward ionic current is normally required and the loss of cell potassium would be proproportionately less.

The properties of the set of channels opened by the opioids can be obtained by the method of subtracting the currents. The results indicate that the potassium channels opened by the opioids are probably not significantly in operation before the drug is applied; equivalent circuits for the cell (Figure 3b) indicate that the agonists open a set of channels having relatively unique properties. The conductance of the channels changes almost immediately with membrane potential, being higher at negative potentials (inward rectification); the inward current is particularly sensitive to barium, being reduced by as little as 1 μM (Williams *et al.*, 1988a). The significance of the inward rectification (in these or other potassium channels) is not well understood, but the conductance increase over the very voltage range at which the agonists are normally working tends to amplify their effects.

We know that a single neurone typically has at least two receptors that can couple to potassium channels; it is possible that each receptor is associated with, or actually comprises, its own channels. This can be readily tested by recording the membrane current from the entire neurone: if a maximal outward current is elicited by an agonist at one receptor type, then a second agonist should still be effective to open 'its own' channels through 'its own' receptor. The experiments depicted in Figure 4 show that this is not the case. The figure shows a recording of the membrane current of a neurone in the rat substantia nigra zona compacta; the concentration of baclofen was increased until it caused a maximal outward current, and at this stage the action of dopamine was occluded. A second example (Figure 4b) shows that noradrenaline and somatostatin open the same channels in a submucous plexus neurone. The outward current evoked by noradrenaline was

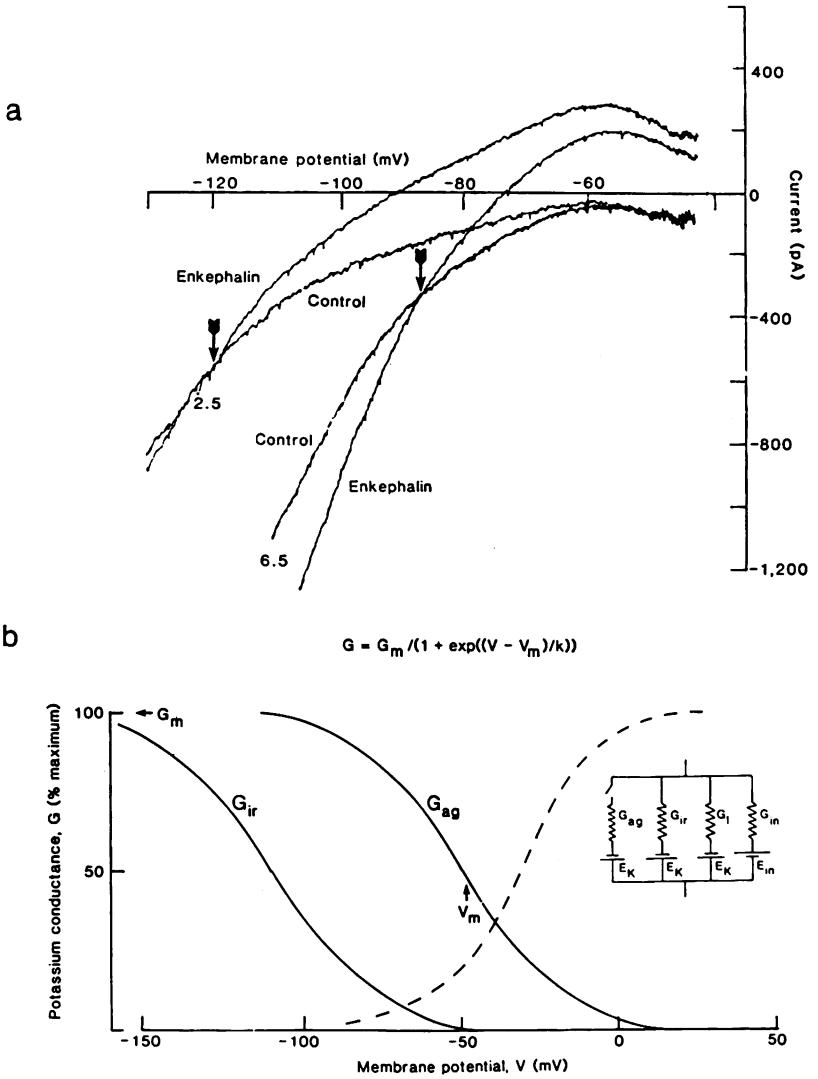

Figure 3 (a) [Met^5]enkephalin increases potassium conductance in a rat locus coeruleus neurone. The left two traces (labelled 2.5) show the membrane currents (I, inward current is downward) recorded as the membrane potential (V) was slowly changed from -130 mV to -45 mV. In control circumstances, the conductance of the cell (dI/dV) steadily falls until the potential reaches about -60 mV. (At -60 mV the net membrane current is close to zero; this would be the resting potential of the cell. The steady-state membrane current is actually still slightly inward at -60 mV, because locus coeruleus neurones fire spontaneously under these conditions. The time-dependent currents underlying the action potential are not seen.) When [Met^5]enkephalin (30 μM) is added, an additional membrane current flows which is large and outward at -60 mV, zero at about -120 mV (arrow), and reverses to inward at more negative potentials. The right pair of traces (labelled 6.5) show the results of repeating the experiment in a higher potassium concentration ($[K]_o$, 6.5 instead of 2.5 mM). The potential at which [Met^5]enkephalin causes no net current (arrow) changes to about -88 mV. In many such experiments it is found that [Met^5]enkephalin (and the other agonists listed in Table 1) cause a membrane current which reverses at a potential (E_{rev}) which is directly related to the logarithm of $[K]_o$. This indicates that the current caused by [Met^5]enkephalin results from an increase in the membrane potassium conductance. (b) Many neurones behave as though they have two potassium conductances operating in the range of voltage between -60 and -130 mV. These are a voltage-independent conductance (G_l) and an inward rectifier conductance (G_{ir}) that increases with hyperpolarization according to $G_{ir} = G_m/(1 + \exp((V - V_m)/k))$ where V_m is the potential at which G_{ir} is half its maximal value (G_m) and k represents the steepness of the voltage-dependence. Agonists such as [Met^5]enkephalin act to increase a potassium conductance (G_{ag}) which is also voltage-dependent, but the potential at which it is half-maximal (V_m, arrow) is closer to the resting potential of the cell. Adapted from Williams *et al.* (1988a).

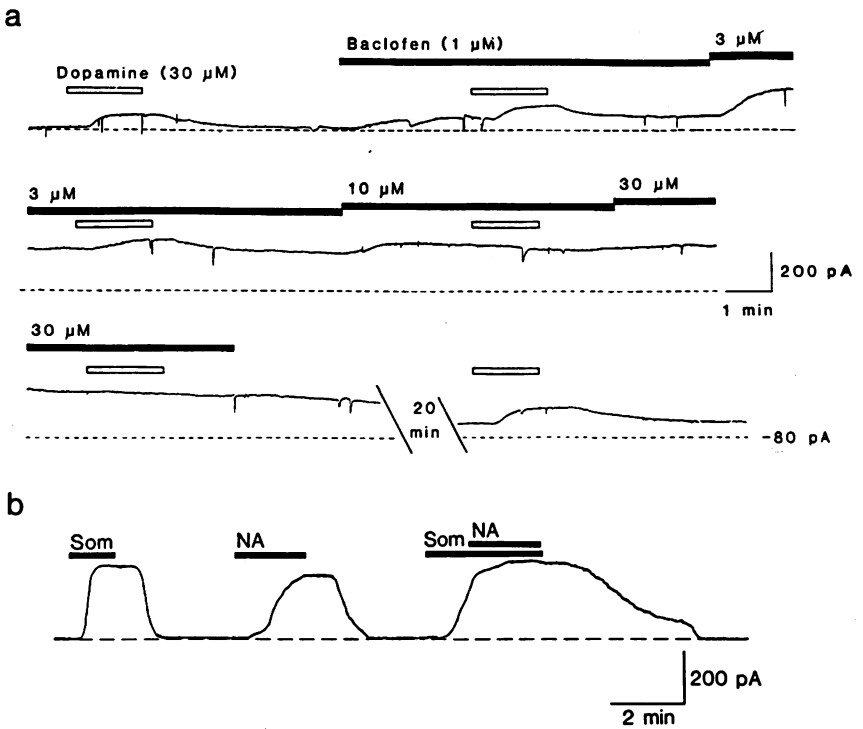

Figure 4 Several agonists increase the same potassium conductance on individual neurones. (a) Membrane current of a rat substantia nigra neurone. Dopamine (open bars) and baclofen (filled bar) were applied. The outward current evoked by baclofen became greater as the concentration was increased; when the baclofen-induced current was maximal, dopamine became ineffective. Holding potential −60 mV. (b) A similar experiment to show that somatostatin (Som, 5 nM) occludes the current caused by noradrenaline (NA, 2 μM) in a neurone of guinea-pig submucous plexus. These agonist concentrations gave maximal currents in this neurone. Holding potential −55 mV. Reproduced, with permission, from Lacey *et al.* (1987) (a) and Surprenant & North (1988) (b).

completely occluded by the outward current caused by somatostatin: a similar result was found with enkephalin. I need hardly remind you that somatostatin, enkephalin and noradrenaline act at distinct receptors: Figure 5 shows the selective antagonism of the hyperpolarizations caused by these agonists when applied to a single submucous plexus neurone.

Receptor-channel coupling

An obvious way in which agonists at distinct receptors might open the same potassium channel is by activating the same second messenger molecule. For all the receptors in the family, agonist binding affinity is reduced by adding guanosine 5′-triphosphate (GTP), and several of the receptors have been co-purified with G-proteins. If GTP hydrolysis is prevented, by providing the cell interior with a non-hydrolyzable derivative such as guanosine 5′-*O*-(3-thiotriphosphate) (GTP-γ-S), then the action of the agonists does not reverse when the application is

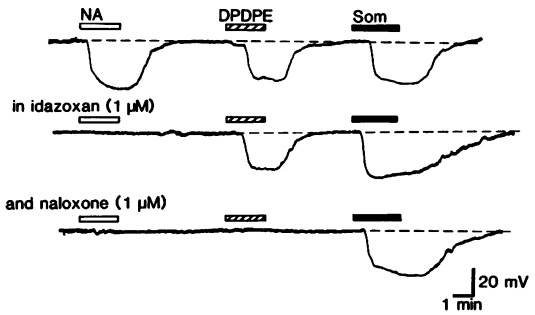

Figure 5 Activation of distinct receptors (α_2-adrenoceptor, δ-opioid and somatostatin) on the same neurone causes hyperpolarization. Three agonists [noradrenaline (NA, 2 μM), Tyr-D-Pen-Gly-Phe-D-Pen (DPDPE, 200 nM) and somatostatin (Som, 5 nM)] caused approximately the same hyperpolarization of this guinea-pig submucous plexus neurone. Idazoxan selectively blocked the action of noradrenaline, and naloxone blocked the effect of the enkephalin analogue. Reproduced, with permission, from Mihara *et al.* (1987a).

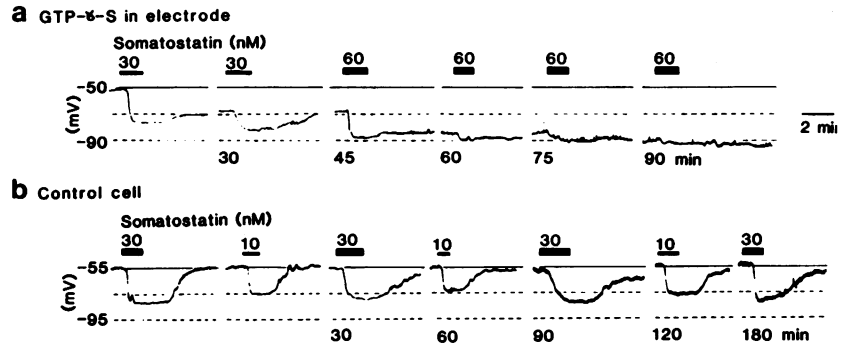

Figure 6 Hydrolysis of guanosine 5'-triphosphate (GTP) is necessary for the termination of the hyperpolarization caused by somatostatin. (a) Intracellular recording from a guinea-pig submucous neurone: the recording electrode contained potassium chloride and guanosine 5'-*O*-(3-thiotriphosphate) (GTP-γ-S, 20 mM), a non-hydrolyzable analogue of GTP. Successive applications of somatostatin caused progressive hyperpolarizations which eventually caused the membrane potential to remain close to the potassium equilibrium potential (about −95 mV, potassium concentration was 5 mM). Times indicate the time after impaling the neurone, during which GTP-γ-S could diffuse into the neurone. (b) A similar experiment in which somatostatin was applied repeatedly to another submucous plexus neurone, when the recording electrode contained only potassium chloride. The effect of the somatostatin remained fully reversible during 3 h of recording. Reproduced, with permission, from Mihara *et al.* (1987a).

discontinued, and the potassium channels remain open (Figure 6). In all the cases tested, the coupling to the potassium channel involves a *Pertussis toxin*-sensitive G-protein (Table 1). The action of the agonists can be prevented by low concentrations of *Pertussis toxin* applied to the cell interior, or by larger concentrations applied for an hour or so to the external surface. In the latter case, the lost response to the agonists can be 'reconstituted' if purified G proteins are included in the recording electrode so that they diffuse into the cell (North *et al.*, 1988).

A second kind of evidence implicates a secondary transducing molecule within the cell; this is the time course of the response to agonist. When the agonist is applied briefly, the channels open after a delay of at least 20–30 ms, commonly more. This contrasts markedly with the delay that precedes the nicotinic action of acetylcholine in the same cell, which can be made less than 1 ms. We have studied this in detail for the action of noradrenaline (Figure 7). The noradrenaline was applied by iontophoresis from a pipette tip situated within a few micrometers of the cell membrane, the application lasted 0.5 ms. Yet the hyperpolarization begins only after 30 ms. A similar application of acetylcholine to the same cell resulted in an almost immediate depolarization, which had completely disappeared before the noradrenaline response peaked.

Synaptic inhibition

The time course of the response is particularly well seen when the agonist is released as transmitter by a synchronous shock to presynaptic nerves. Focal stimulation of the nucleus raphe dorsalis in a brain slice releases 5-HT to cause synaptic potentials in adjacent neurones (Figure 8). The noradrenaline-containing sympathetic fibres can also be stimulated as they enter the submucous plexus; the released noradrenaline causes an i.p.s.p. Note the similarity in the time courses of the two synaptic potentials; the difference in decay rate may reflect differences in diffusion and reuptake mechanisms, since the brain slice offers a greater barrier to diffusion and hence opportunity for uptake than does the isolated ganglion of the submucous plexus (Surprenant & Williams, 1987).

Currents through single potassium channels

These responses are therefore considerably slower than those, such as the nicotinic action of acetylcholine, in which the receptor is itself a channel. But they are faster than many responses associated with diffusion of second messenger molecules throughout the cell cytoplasm (see Hartzell, 1981; North, 1986b). This suggests the possibility that the association between receptor and channel is fairly intimate. This has been shown directly by recording from single potassium channels (Miyake *et al.*, 1989). Figure 9 shows the currents through single channels in the membrane of a rat locus coeruleus neurone. The neurones were dissociated from neonatal rats, which we knew from a series of preliminary experiments to give robust hyperpolarizations when μ-receptor agonists were applied (Miyake *et al.*, 1989). The

Table 1 Drug receptors and increases in potassium conductances: evidence for involvement of G-protein comes either from block by pertussis toxin, or activation by GTP-γ-S

Agonist (receptor)	*Tissue*	*G-protein*	*Synaptic potential*	*Reference*
Acetylcholine (M?)	Frog heart	—	Yes	del Castillo & Katz, 1955
Acetylcholine (M?)	Guinea-pig cardiac muscle	—	—	Soejima & Noma, 1984
Acetylcholine (M?)	Guinea-pig cardiac muscle	Yes	—	Pfaffinger *et al.*, 1985
Acetylcholine (M?)	Guinea-pig cardiac muscle	Yes	—	Breitwieser & Szabo, 1985
Acetylcholine (M?)	Guinea-pig cardiac muscle	Yes	—	Yatani *et al.*, 1987a
Acetylcholine (M?)	*Helix aspersa* ganglion	—	Yes	Tauc & Gerschenfeld, 1962
Acetylcholine (M?)	*Aplysia* pleural ganglion	—	—	Kehoe, 1972
Acetylcholine (M?)	*Aplysia* abdominal ganglion	Yes	—	Sasaki & Sato, 1987
Acetylcholine (M?)	Mudpuppy cardiac ganglion	—	Yes	Hartzell *et al.*, 1977
Acetylcholine (M?)	Frog sympathetic C cells	—	Yes	Dodd & Horn, 1983
Acetylcholine (M?)	Cultured GH_3 pituitary cells	Yes	—	Yatani *et al.*, 1987b
Acetylcholine (M_2)	Rat nucleus parabrachialis	—	—	Egan & North, 1986b
Acetylcholine (M_2)	Rat thalamus reticularis	—	—	McCormick & Prince, 1986
Acetylcholine (M_1)	Guinea-pig submucous plexus	Yes	Yes	Mihara & Nishi, 1989
Noradrenaline (α_2)	Guinea-pig submucous plexus	—	Yes	North & Surprenant, 1985
Noradrenaline (α_2)	Guinea-pig submucous plexus	Yes	Yes	Surprenant & North, 1988
Noradrenaline (α_2)	Rat nucleus locus coeruleus	—	Yes	Egan *et al.*, 1983
Noradrenaline (α_2)	Rat nucleus locus coeruleus	—	—	Williams *et al.*, 1985
Noradrenaline (α_2)	Rat nucleus locus coeruleus	Yes	—	Aghajanian & Wang, 1986
Noradrenaline (α_2)	Rat substantia gelatinosa	—	—	North & Yoshimura, 1984
Noradrenaline (α_2)	Guinea-pig myenteric plexus	—	—	Morita & North, 1981
Noradrenaline (α_2)	Rat preganglionic sympathetic neurones	—	Yes	Yoshimura *et al.*, 1987
Enkephalin (μ)	Guinea-pig locus coeruleus	—	—	Pepper & Henderson, 1980
Enkephalin (μ)	Guinea-pig myenteric plexus	—	—	Morita & North, 1982
Enkephalin (μ)	Rat hippocampus interneurones	—	—	Madison & Nicholl, 1987
Enkephalin (μ)	Rat substantia gelatinosa	—	—	Yoshimura & North, 1983
Enkephalin (μ)	Mouse dorsal root ganglion	—	—	Werz & Macdonald, 1983
Enkephalin (μ)	Rat nucleus locus coeruleus	—	—	Williams & North, 1984
Enkephalin (μ)	Rat nucleus locus coeruleus	Yes	—	North *et al.*, 1987
Enkephalin (μ)	Rat nucleus locus coeruleus	Yes	—	Aghajanian & Wang, 1986
Enkephalin (μ)	Rat nucleus parabrachialis	—	—	Christie & North, 1988
Enkephalin (μ)	Guinea-pig hypothalamus	—	—	Charpak *et al.*, 1988
Enkephalin (δ)	Guinea-pig submucous plexus	Yes	—	North *et al.*, 1987
Enkephalin (δ)	Guinea-pig submucous plexus	—	—	Mihara & North, 1986
γ-Aminobutyric acid ($GABA_B$)	Rat hippocampus pyramidal cells	—	—	Gahwiler & Brown, 1985
γ-Aminobutyric acid ($GABA_B$)	Rat hippocampus pyramidal cells	Yes	—	Newberry & Nicoll, 1985
γ-Aminobutyric acid ($GABA_B$)	Rat hippocampus pyramidal cells	—	Yes	Newberry & Nicoll, 1984
γ-Aminobutyric acid ($GABA_B$)	Rat hippocampus pyramidal cells	Yes	—	Andrade *et al.*, 1986
γ-Aminobutyric acid ($GABA_B$)	Rat hippocampus pyramidal cells	Yes	Yes	Thalmann, 1989
γ-Aminobutyric acid ($GABA_B$)	Rat nucleus substantia nigra	Yes	—	Lacey *et al.*, 1988
γ-Aminobutyric acid ($GABA_B$)	Rat nucleus parabrachialis	—	—	Christie & North, 1988a
γ-Aminobutyric acid ($GABA_B$)	Rat frontal neocortex	Yes	—	Howe *et al.*, 1987
γ-Aminobutyric acid ($GABA_B$)	Rat nucleus locus coeruleus	—	—	Osmanovic & Shefner, 1987
γ-Aminobutyric acid ($GABA_B$)	Rat nucleus dorsal raphe	—	—	Colmers & Williams, 1988
Adenosine (A_1)	Mouse striatal cells in culture	—	—	Trussell & Jackson, 1985
Adenosine (A_1)	Guinea-pig cardiac cells	—	—	Kurachi *et al.*, 1986

Table 1—*continued*

Agonist (receptor)	*Tissue*	*G-protein*	*Synaptic potential*	*Reference*
Somatostatin	Guinea-pig myenteric plexus	—	—	Katayama & North, 1980
Somatostatin	Cultured GH_3 pituitary cells	—	—	Yamashita *et al.*, 1986
Somatostatin	Guinea-pig submucous plexus	—	Yes	Mihara *et al.*, 1987a
Somatostatin	Guinea-pig submucous plexus	Yes	—	Mihara *et al.*, 1987b
Somatostatin	Cultured GH_3 pituitary cells	Yes	—	Yatani *et al.*, 1987b
Somatostatin	Rat nucleus locus coeruleus	—	—	Williams *et al.*, 1987
Somatostatin	Rat nucleus locus coeruleus	Yes	—	Inoue *et al.*, 1988
Somatostatin	Cultured AtT-20 cells	Yes	—	Pennefather *et al.*, 1988
Somatostatin	Rat somatotrophs in culture	—	—	Chen *et al.*, 1989
5-hydroxytryptamine	*Aplysia* buccal and abdominal, and *Helix* pallial ganglion	—	—	Gerschenfeld & Paupardin-Tritsch, 1974
5-hydroxytryptamine	*Aplysia* R15 neurone	—	—	Benson & Levitan, 1983
5-hydroxytryptamine	Guinea-pig myenteric plexus	—	—	Johnson *et al.*, 1980
5-hydroxytryptamine	Rat nucleus dorsal raphe	—	Yes	Yoshimura & Higashi, 1985
5-hydroxytryptamine ($5HT_{1A}$)	Guinea-pig myenteric plexus	—	—	Galligan *et al.*, 1988
5-hydroxytryptamine ($5HT_{1A}$)	Rat nucleus dorsal raphe	—	—	Sprouse & Aghajanian, 1987
5-hydroxytryptamine ($5HT_{1A}$)	Rat nucleus dorsal raphe	Yes	Yes	Williams *et al.*, 1988b
5-hydroxytryptamine ($5HT_{1A}$)	Guinea-pig prepositus hypoglossi	—	Yes	Bobker & Williams, unpub.
5-hydroxytryptamine ($5HT_{1A}$)	Rat hippocampus pyramidal cells	Yes	—	Andrade & Nicoll, 1987
5-hydroxytryptamine ($5HT_{1A}$)	Rat hippocampus pyramidal cells	—	—	Colino & Halliwell, 1987
Dopamine	*Aplysia* pleural ganglion	—	—	Kehoe, 1972
Dopamine	*Aplysia* pleural ganglion	—	—	Ascher, 1972
Dopamine	*Aplysia* abdominal ganglion	Yes	—	Sasaki & Sato, 1987
Dopamine (D_2)	Human pituitary adenoma	—	—	Israel *et al.*, 1985
Dopamine (D_2)	Rat intermediate pituitary	—	Yes	MacVicar & Pittman, 1986
Dopamine (D_2)	Rat pituitary corticotrophs	—	—	Stack & Surprenant, 1988
Dopamine (D_2)	Rat nucleus substantia nigra	—	—	Lacey *et al.*, 1987
Dopamine (D_2)	Rat nucleus substantia nigra	Yes	—	Innis & Aghajanian, 1987
Galanin	Mudpuppy cardiac ganglion	—	—	Konopka *et al.*, 1989
Histamine	*Aplysia* neurones	Yes	—	Sasaki & Sato, 1987

direction of current flow is inward in these experiments, becoming larger when the electrical potential difference across the membrane patch is increased.

The kinetic behaviour of the channels allows one to resolve certain states. The channels tend to close for long periods of time (several seconds to minutes) and then begin to chatter in a 'period of activity' of similar duration. Within such periods of activity, the channels behave as though they have a single open state and two closed states. The 'periods of activity' are more frequent when the concentration of enkephalin is increased, and within them the open state of the channel appears to be stabilized by higher enkephalin concentrations. This rather surprising finding that the duration of an open state is influenced by the agonist concentration might be interpreted to indicate that the opening mechanism involves the cooperative action of several molecules or subunits, as would be expected if the channel is formed from several closely similar or identical subunits (see below).

The records in Figure 9 were obtained in the 'on-cell' or 'cell-attached' configuration in which the tight seal between the recording pipette and the cell membrane effectively isolates the compartment containing the potassium channel under study from the remainder of the cell membrane. For channel openings to be seen, it was necessary to have the opioid agonist in the recording electrode; the opioid agonist was not effective to open the potassium channels when it was applied to the remainder of the cell. We conclude from this experiment that the receptor and

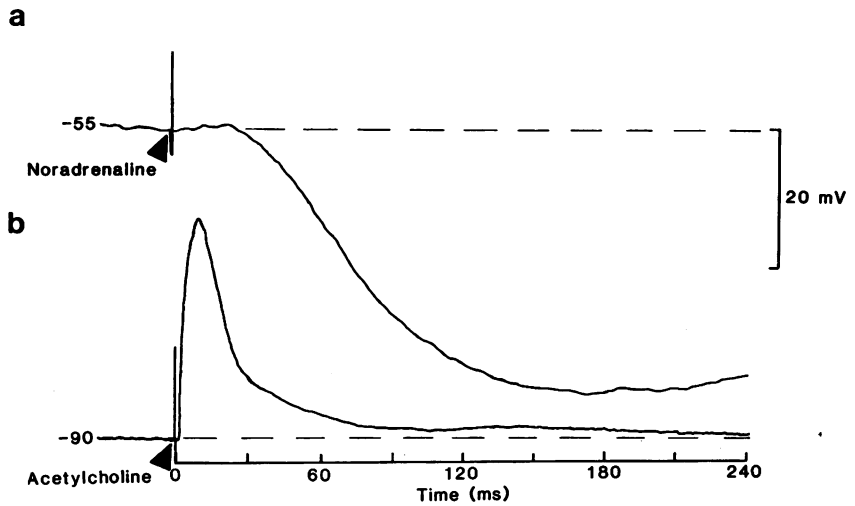

Figure 7 Potassium conductance increase evoked by noradrenaline has a latency of tens of milliseconds. Records show intracellular recording of membrane potential from a guinea-pig submucous plexus neurone. (a) Noradrenaline was applied by iontophoresis (triangle). The neurone was at the resting potential, −55 mV. The iontophoresis pipette contained noradrenaline (10 mM) and its tip was positioned within 5 μm of the neuronal membrane: a rectangular current pulse of 0.5 ms duration was used to eject noradrenaline. The hyperpolarization begins with a latency of at least 30 ms and reaches its peak in 180 ms. (b) The pipette was then removed, emptied, refilled with acetylcholine (1 M) and repositioned. The membrane potential was held at −90 mV. Ejection of acetylcholine (0.5 ms pulse) caused a depolarization of very short latency. This depolarization is blocked by hexamethonium (not shown) and is presumed to result from activation of nicotinic receptors. Unpublished observations of A. Surprenant.

the ion channel are located close to each other, within the few square micrometres of membrane isolated by the pipette tip. We interpret these findings to indicate that a freely diffusible second messenger is probably not involved in the transduction from these receptors to the potassium channel. We found that channels of very similar properties were also opened by α_2-receptor agonists and somatostatin on these cells (Miyake *et al.*, 1989). In these respects, the situation seems very similar to that first described by Soejima & Noma (1984) for the action of acetylcholine in the heart.

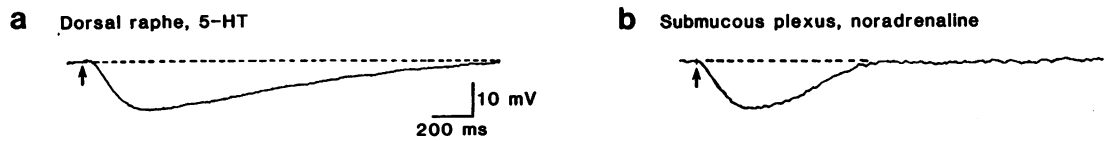

Figure 8 Synaptic inhibition of mammalian neurones mediated by potassium conductance increase. (a) Hyperpolarizing synaptic potential (i.p.s.p.) recorded from a dorsal raphe neurone in a brain slice, following a single electrical stimulus to the surface of the slice (arrow). Fast postsynaptic potentials which normally precede the i.p.s.p. have been blocked by DL-2-amino-5-phosphonovaleric acid (30 μM), 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline (10 μM) and picrotoxin (100 μM). The i.p.s.p. arises with a latency of about 30 ms (not resolved at this recording speed) and reaches its peak in about 200 ms. This synaptic potential is blocked by antagonists at 5-HT_{1A} receptors. (b) A similar synaptic potential in a neurone of the guinea-pig submucous plexus. In this case, the transmitter (noradrenaline) was released by an electrical stimulus to the sympathetic nerve fibres entering the ganglion. Hexamethonium (100 μM) was present to block the nicotinic synaptic depolarization resulting from concomitant stimulation of cholinergic fibres. Note the similarity of the rising phases of the two potentials. The decay of the synaptic potential in the dorsal raphe is thought to be prolonged because of barriers to diffusion in the brain slice which are absent in the submucous ganglia (see Surprenant & Williams, 1987). Unpublished observations of J.T. Williams (a) and A. Surprenant (b).

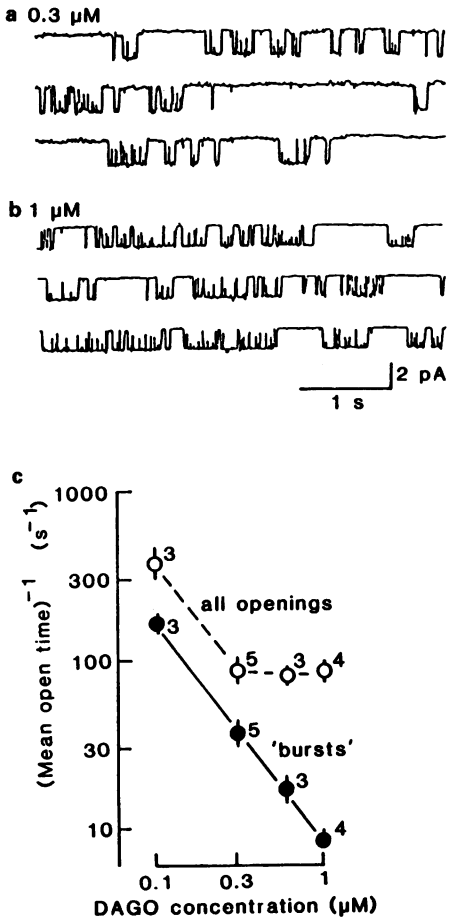

Figure 9 Currents through single potassium channels in the membrane of a rat locus coeruleus neurone. (a & b) Single channel currents: the recording electrode contained (among other things) 150 mM potassium gluconate and the enkephalin analogue Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO) at 300 nM (a) and 1 μM (b). The electrode was used to make a cell-attached (on-cell) recording from the neurone; the remainder of the neurone, not isolated by the patch electrode, was bathed in a normal physiological saline solution. The potential across the membrane patch (determined later by breaking the membrane and correcting for junction potentials) was about −46 mV. Thus, potassium currents are inward (downward) when the channel opens. (c) Mean open time increases with agonist concentration: (○) mean open times computed from all openings and closings; (●) mean burst duration (open times computed when brief closures (<4 ms) were ignored). Points show mean value (±s.e.mean) for the number of patches indicated. A relatively small change in agonist concentration has a large effect on the burst duration. This would be consistent with cooperativity in the activation of the channel by G-protein. Reproduced, with permission, from Miyake *et al.* (1989).

Divergence after the receptor

When γ-aminobutyric acid (GABA) binds to its receptor, and a chloride channel opens, and inhibition occurs, only a single molecule is primarily involved. The interpolation of a molecule *between* receptor and channel provides a means for diversification of the action of the agonist. In fact, opening potassium channels is rarely the only action that results from activation of receptors in this *family*. Let me give two examples.

In the same cell the receptors can couple to different effectors: they may use the same G-protein. This should come as no surprise because we know that all these receptors (Table 1) will inhibit the activity of adenylate cyclase through a *Pertussis*-sensitive G_i protein. We have found that noradrenaline will not only increase the potassium conductance of submucous neurones, but will inhibit calcium currents, even in the same cell. This depression of the 'calcium' current results from activation of α_2 receptors, and also requires a *Pertussis toxin*-sensitive G-protein (North *et al.*, 1988). In other words, at least two ion channels can be affected in the same neurone.

Second, the same receptors also appear able to couple to more than one G-protein, and thus to distinct effectors, in different neurones (Figure 10). For example, agonists at the cardiac type of M_2 receptor on rat parabrachial neurones lead to an opening of potassium channels and inhibition of the type that I have described (Christie & North, 1988a); the typical outward currents are shown in Figure 10b. On locus coeruleus cells, μ-opioids open the potassium channels (Williams *et al.*, 1982; North & Williams, 1985)

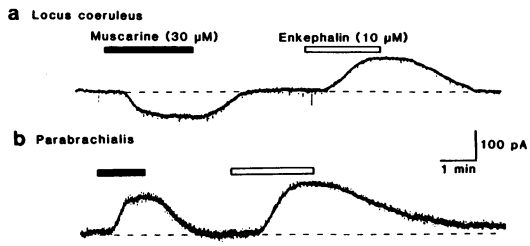

Figure 10 Contrasting actions of muscarine (acting at M_2-receptors) on neurones of the locus coeruleus (a) and parabrachialis (b) of the rat. In both nuclei [Met^5]enkephalin causes an outward potassium current, by acting at μ-opioid receptors. In the parabrachialis neurone (b), muscarine also causes an outward current; this results from opening the same population of potassium channels (see Christie & North, 1988). In the locus coeruleus neurone (a), muscarine causes an inward rather than outward current. The type of the muscarinic receptor on the two cell types can not be distinguished by AFDX-116, pirenzepine or hexahydrosiladifenidol. Unpublished observations of M.J. Christie.

but muscarine instead causes an inward current (Figure 10a) (Egan & North, 1986a). We know that the locus coeruleus neurone has the *Pertussis*-sensitive G-protein and the potassium channels, because they are used by the μ-receptors (Aghajanian & Wang, 1986; J.T. Williams & M.J. Christie, unpublished observations); and we also have evidence that the muscarinic receptor on these cells is the same (cardiac M_2) as those on the parabrachial cells (more precisely, it can not be distinguished by the antagonists pirenzepine, AFDX-116 and hexahydrosiladifenidol; Christie & North, 1988b) but the excitatory action of muscarine in the locus coeruleus is resistant to *Pertussis toxin*, suggesting that the receptor couples to a different G protein (M.J. Christie & R.A. North, unpublished observations.

The overall picture that emerges is illustrated schematically in Figure 11. The receptor might be any member of our receptor *family* (Table 1). Almost all the main transmitters are able, through one of their receptor types, to couple to potassium channels. It remains unclear what might be the functional role of the coupling to the other effectors. If a neurone is inhibited, it might also be appropriate for it to reduce slightly the level of calcium in the cell body as well as the level of cyclic AMP—both would tend to slow gene transcription and thus reduce the turnover of essential molecules, such as transmitters, receptors and channels. It is the inhibition itself that has been the primary theme of my lecture, and there is abundant evidence that the functional role here is synaptic inhibition. Of course many drugs in current therapeutic use act as agonists and antagonists at this *family* of receptors.

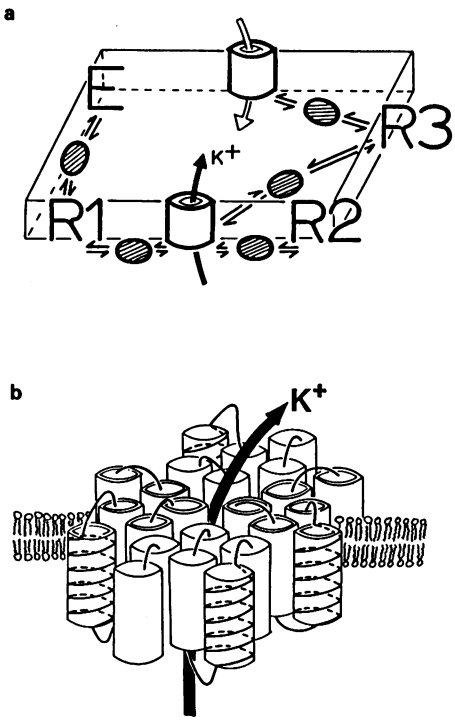

Figure 11 (a) Diagram to illustrate the way in which activation of several membrane receptors might open the same potassium channel. R1, R2 and R3 represent distinct receptors (for example, α_2-adrenoceptor, δ-opioid and somatostatin in Figure 5). The hatched egg-like symbols represent G-proteins (same or different species). The channel at the rear, and E, represent other membrane channels (e.g. calcium channels) and other effectors (e.g. adenylate cyclase) which might be affected by activated G-proteins. (b) Schematic representation of possible membrane topology of a rat brain potassium chanel (RBK1, see Christie *et al.*, 1989). Only transmembrane regions are shown, which are postulated to form pseudocylindrical α-helices. A single subunit is thought to contain six (or seven) membrane spanning regions. Analogy with the voltage-dependent sodium channel (Noda *et al.*, 1984) suggests that four such subunits may together form a potassium channel with a central conducting pore.

The molecules of inhibition

Let me close by making some remarks about the structure of the molecules themselves. The detailed structure of the potassium channel in question is unclear. However, in a collaboration with molecular biologists at the Vollum Institute we have recently isolated from the rat hippocampus a cDNA clone which we call RBK-1 (Christie *et al.*, 1989; see also Stuhmer *et al.*, 1988). The RBK-1 sequence was subcloned into a plasmid expression vector, and the mRNA transcripts synthesized *in vitro* were injected into *Xenopus* oocytes. One to four days later the oocytes expressed very large potassium currents that were not seen at all in uninjected cells. The predicted protein comprises 495 amino acids, has six or seven hydrophobic membrane spanning regions, including one with seven equally spaced positively charged residues that presumably serves as the voltage sensor (Figure 11b). This potassium channel protein is quite homologous to the central core region of the expected translation products of the *Shaker* locus of *Drosophila*, which also form potassium channels (Papazian *et al.*, 1987). It is our expectation that our isolated clone encodes the formation of one subunit, and that perhaps four such subunits (not necessarily identical) together form the potassium channel (Figure 11b). The details of the potassium conductance of oocytes injected with RBK-1 mRNA indicate that it is not likely to be the one which is

regulated by G-proteins; it is opened by depolarization rather than by hyperpolarization and has features of both the delayed rectifier and the A-types of potassium conductance. We are now working to isolate homologous clones in the hope of discovering more about potassium channels in general and perhaps finding the structure of the channel gated by activated G-protein that is used to inhibit neurones by the receptors of Table 1.

Finally, what about the receptors of this family? The structure of several has now been determined by molecular cloning [muscarinic M_2 (Peralta *et al.*, 1987), 5-HT_{1A} (Kobilka *et al.*, 1987a), platelet α_2 (Kobilka *et al.*, 1987b), kidney α_2 (Regan *et al.*, 1988), dopamine D_2 (Bunzow *et al.*, 1988)]. They all belong to the class of receptors having seven transmembrane regions, and share considerable homology with rhodopsin; those that couple to G_i preferentially seem to have large cytoplasmic loops (intracellular loop 3, linking transmembrane segments 5 and 6) and short C terminals. Rapid progress is to be expected in the determination of the tertiary structure of these and other receptors in this class.

This image of the receptor is rather different from that presented by Gaddum in 1926; his was a more mysterious view, based heavily on inference and the Law of Mass Action. Our affair with these receptors deepens daily. We shall soon know the roles of particular amino acids within them, and much more about the disposition of the individual family members. They seem unclothed before us, every bony peptide bond and proline kink intimately revealed. William Hazlitt wrote 'Though familiarity may not breed contempt, it takes the edge off admiration'. The drug receptors involved in neuronal inhibition are increasingly familiar to us, and we may admire them less. But the admiration for John Gaddum remains: without him we may never have set forth on this great romance.

The work described in this lecture has been a long term collaborative effort with many colleagues. Macdonald Christie, Mike Lacey, Annmarie Surprenant and John Williams contributed particularly to the experimental work illustrated; I am greatly indebted to them and to other past and present coworkers. The research has been supported by grants DK32979, DA03160, DA03161 and MH40416 from the U.S. Department of Health and Human Services.

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