RÉSUMÉ

No neck

THE myosin head is divided into two domains, one containing the ATPase and binding sites, the other (the neck that connects the head to the filament trunk) consisting of a long α-helix to which the two light chains are attached. This neck is thought to convert the movement of the jaws around the ATPase site into the power stroke that drives the head along the actin filament. But things may not be so simple. S. Itakura *et al.* (*Biochem. biophys. Res. Commun.* **196**, 1504–1510; 1993) have prepared a neckless myosin head, and coupled a reactive cysteine to its neck end, as well as to wild-type heads; this allows the heads to be attached to a surface in a defined orientation, by way of an avidin–biotin link. The surprise is that the mutant heads apparently propel actin filaments along almost as well as the wild type.

Bang on course

ASTRONOMERS and others with a relish for celestial fireworks will be pleased to hear that Shoemaker–Levy 9, known as the 'string of pearls' comet, has definitely made a date with Jupiter. The latest accurate orbital parameters have been computed by J. V. Scotti and T. Metcalfe for nine of the individual cometary nuclei, from images obtained with the Spacewatch telescope between March and July this year (before the comet disappeared behind the Sun), and are published in *Minor Planet Circulars* of 29 November. Armed with advance notice of these computations, B. G. Marsden (*IAU Circ. No.* 5893 (22 November 1993) has pinned down the expected collision times to within a couple of hours: nucleus 17 will hit the far side of the planet at 1994 July 18.7 (Universal Time); nucleus 15 at July 19.1; 14 at 19.6; 12 at 20.2; 11 at 20.9; 7 at 21.6; 6 at 22.1; 5 at 22.7; and finally, to round off five remarkable days in Jupiter's recent history, nucleus 1 is due to plummet into the planet at July 23.2.

Small advantage

VOLES are not ruminants. So why is their digestive efficiency so much higher than, say, that of cows? The answer, according to W. B. Lee and D. C. Houston, lies in their ability to chew their food — often plant leaf material — into very small particles, so giving their digestive enzymes a head start. As part of a wider study (*J. Zool.* **231**, 301–309; 1993), Lee and Houston put voles on high-fibre and low-fibre diets, and examined the resulting size of food particles; they also looked at tooth microstructure. Voles can grind exceedingly fine, seemingly a consequence of their tooth design, and tooth wear alters with diet. This, the authors speculate, may result in maintenance of high chewing — and digestive — efficiency as food quality changes.

MOLECULAR PHARMACOLOGY

The binding issue

D. Colquhoun and M. Farrant

ON page 565 of this issue[1], Amin and Weiss show that certain mutations in the amino-terminal region of a $GABA_A$-receptor subunit reduce the effectiveness (potency) of the agonist, GABA (γ-aminobutyric acid, the major inhibitory neurotransmitter in the brain). But is it the ability of GABA to bind that is reduced, or the ability of GABA to open the channel once bound? If it is the former, then the mutated amino acids may be part of the agonist binding site, so the answer to this question is important. Amin and Weiss have addressed the problem clearly, and give reasons for thinking that it is mainly affinity that is reduced. The distinction is not an easy one, however, and has often been misunderstood. We are now seeing the painful rediscovery by molecular biologists of a standard bit of 1950s pharmacology.

The unthinking reaction to the problem is "If you want to know whether binding affinity is reduced then do a binding experiment". But consideration of even the simplest mechanism for agonist action (see, for example, ref. 2) shows that the binding of an agonist, as measured in a binding experiment, reflects not only its ability to bind initially to the receptor, but also its ability to open the channel once it is bound. The second step, the isomerization between shut and open states both of which have agonist bound, is referred to as 'gating' in the ion-channel literature. This characteristic of binding measurements is expected generally, because of the physical principle of reciprocity (see, for instance, ref. 3): *binding affects gating so gating will affect binding*.

Distinguishing between the effects of mutation on gating and on binding is exactly the same problem as that which led Stephenson[4], in 1956, to distinguish between effects on agonist efficacy and effects on affinity; he, of course, was thinking of effects of changes in agonist structure rather than effects of changes in receptor structure, but the principles are the same. Stephenson showed that if the agonist has high efficacy (that is, in the present context, more than 95 per cent or so of channels are openable by high agonist concentration), changes in affinity were quite indistinguishable from changes in efficacy (gating) on the basis of equilibrium concentration–response curves (increasing either causes a parallel shift to the left with a log concentration scale).

It is because of these problems that most investigations of structure–function relationships in ion channels (such as the now classical study on the effects of rings of charges on ion permeation[5]) have, wisely, focused on effects of mutations on the characteristics of the channel while it is open, which are easier to interpret than effects on binding and gating.

Although these principles have been established since 1956, the history of attempts to distinguish experimentally between affinity and efficacy has been tortuous. The classical methods are unlikely to work, because they too neglect the principle of reciprocity[6]. At this point, the patch clamp comes to the rescue. The information that can be obtained from single-channel recordings allows far more detailed investigations of mechanism than is possible in other systems such as enzymes. This gives ion channels a real advantage in investigations of the structure–activity relationships of proteins (though it is not yet possible to obtain detailed three-dimensional structures of normal and mutated ion channels, which can be done with enzymes).

Measurements of the fine structure of channel openings can, in favourable cases, allow separation of affinity and efficacy (see ref. 7). It is this approach that Amin and Weiss have used. They suggest, on the basis of single-channel data yet to be published, that GABA is an agonist of relatively low efficacy; the open–shut equilibrium constant is estimated to be around 4 — that is, only 80 per cent of channels are openable (in contrast, the equilibrium constant of the nicotinic acetylcholine receptor is around 40, so 98 per cent of channels are openable). If they are right then, in this case, any substantial effect of the mutations on gating would have resulted in a reduction of the maximum response, which was not observed (maxima are of course not easy to estimate precisely, because of problems such as desensitization, but that is another question). The authors therefore conclude that the mutations have probably reduced the initial binding of GABA, and that therefore, with a bit of luck anyway, the mutated residues may have been in the agonist binding regions of the protein.

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