Genetic approaches to study of anaesthesia

Bashir A. Mir and K. S. Krishnan

Molecular Biology Unit, Tata Institute of Fundamental Research, Homi Bhabha Road, Colaba, Bombay 400 005 India

The molecular mechanisms of general anaesthesia are not known. Over the years many different approaches to this problem have failed to identify the molecular target(s) of anaesthetics with certainty. Quite recently, a few mutants have been isolated in *Drosophila melanogaster* and *Caenorhabditis elegans* which deviate from the Meyer–Overton rule and show an altered response to different anaesthetics. In this review we assess the relevance of this newly emerging genetic approach in understanding the molecular mechanisms of anaesthesia.

UNDERSTANDING the molecular and cellular mechanisms underlying drug action is central to drug design. Progress towards such an understanding has been aided by studies of structure–activity relationships. In the case of phenothiazines, for example, nearly a hundred closely related compounds are major tranquilizers and it has been possible to design improvement in these molecules by analysing their action on the basis of their conserved chemical structures.[1] Despite nearly a hundred and fifty years of clinical use, inhalational general anaesthetics defy such an analysis. These agents, which induce reversible loss of consciousness without adversely affecting vital functions, are a widely diverse class of molecules (Figure 1) with hardly any chemical similarity. Hence, approaches based on structure-activity relationship have not been useful in defining the target(s) of anaesthetic action. The state of anaesthesia is defined at the level of the whole animal. Virtually every aspect of nervous system function is depressed. As yet, it is not clearly known which region(s) of the brain or which cellular processes are predominantly affected.

Since general anaesthesia is most widely used in pain management during surgery, an understanding of the molecular mechanisms underlying this phenomenon is an important concern for medical as well as basic sciences. In this review we will attempt to bring together some of the interesting observations regarding general anaesthesia and assess critically the relevance of an emerging genetic approach which has the potential to identify molecules involved in anaesthetic response, from studies at the level of whole animal.

Potency and Meyer–Overton rule

The potency of general anaesthetics has a linear correlation to their hydrophobicity. This was recognized by Meyer and Overton[2–4] well over a hundred years ago. This principle is applicable to all the cases of volatile general anaesthetics (Figure 2) and organisms studied. A high olive oil/gas partition coefficient reflects high hydrophobicity and *vice versa*; MAC is a measure of potency, and the lower the MAC, the more potent is the anaesthetic agent. The correlation holds good when hydrophobicity is determined by olive oil/water partition coefficient, octanol/water partition coefficient[5] or for that matter total lipid/water partition coefficient. A corollary to this is that potency is independent of the size or chemical groups in an anaesthetic molecule and the target site is amphiphilic[5,6]. It is also interesting to note that the product of anaesthetic partial pressure and oil/gas partition coefficient, which gives the concentration of anaesthetic agent at the site of action, varies only slightly from species to species (Table 1). Various attempts have been made to correlate anaesthetic potency with other physical properties of anaesthetic molecule[7–9] but none show the closeness of fit that is observed with oil/gas partition coefficient.

Cut-off effect

Study of anaesthetic effects of homologous series of alkanes and alcohols shows that the higher members of the series are less potent or even nonanaesthetic. This has been termed as the 'cut-off effect'[10]. The fact that the higher homologues of these series are bulky and also have low aqueous solubilities[11] probably explains their inability to reach the target as well as fit in it and consequently their low potency.

Pressure reversal of anaesthesia

A well-known result in anaesthesiology is that the anaesthetic effect of all known agents can be reversed by hyperbaric conditions of the order of 150 atm[12,13]. How pressure exerts an antagonistic effect on anaesthesia is itself an interesting question. Suggestions have been made that anaesthesia results in a volume increase of the membranes and at higher pressures anaesthetic molecules are squeezed out from the site, thus reducing the volume back to normal (critical volume hypothesis). At the same time several results about the expansion of membranes and volume changes are open to discussion because the fractional changes are too miniscule[5,14].

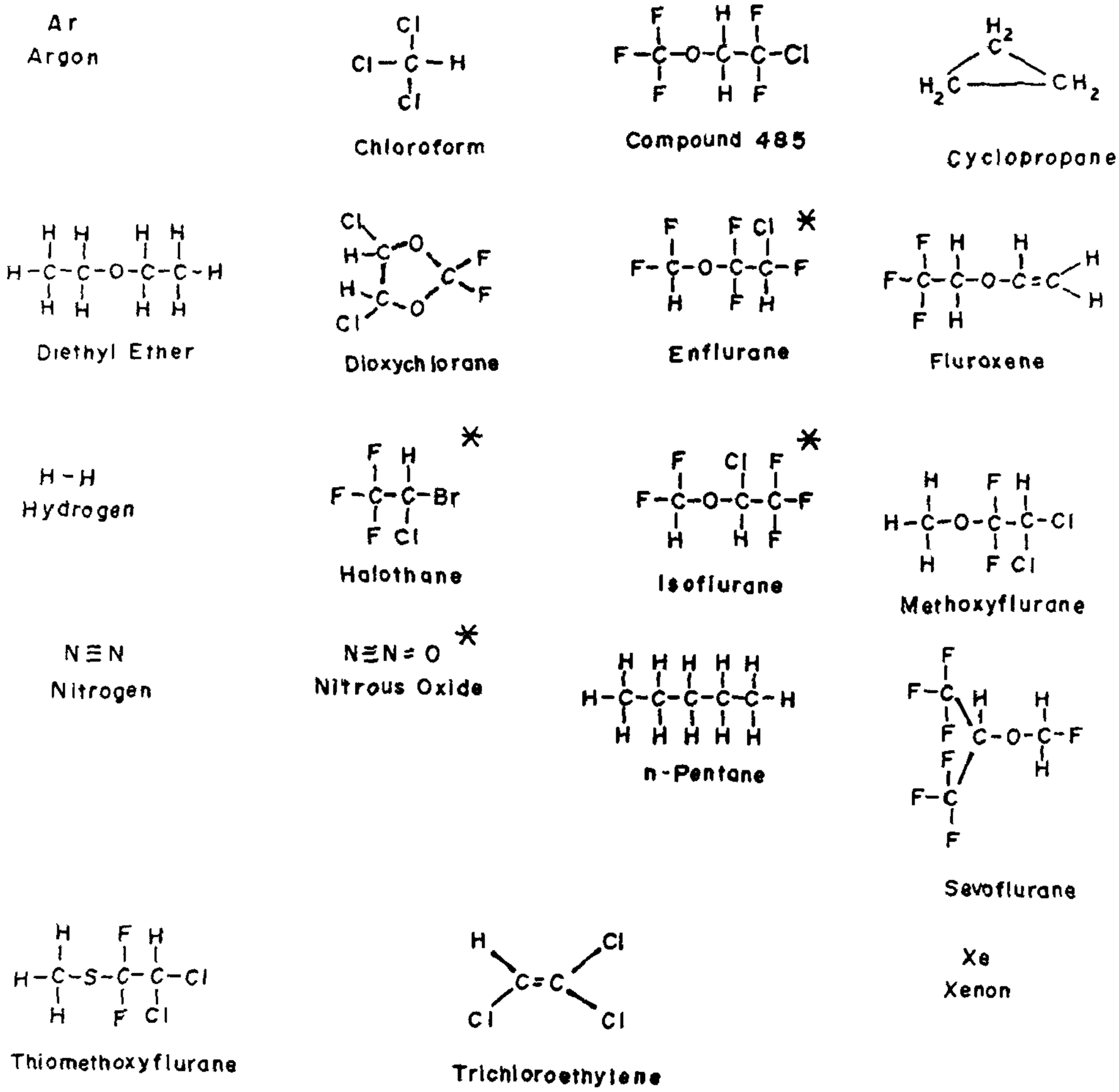


Figure 1. Anaesthetic agents. *Denotes agents in clinical use (modified from ref. 70).

Table 1. Oil/gas partition coefficients and potencies of inhaled anaesthetics in dogs, humans, mice and *Drosophila*

	Oil/gas partition coefficient		Dogs		Humans		Mice		*Drosophila*	
Anaesthetic	37°C	22°C	MAC (atm)	MAC × oil/gas (atm)	MAC (atm)	MAC × oil/gas (atm)	*RR ED_{50} (atm)	*RR ED_{50} × oil/gas (atm)	ED_{50} (atm)	ED_{50} × oil/gas (atm)
Methoxyflurane	970[b]	1790	0 0023[c]	2 23	0 0016[d]	1 55	0.0023[e]	2 23	0 00140	2.51
Chloroform	265[b]	840	0 0077[f]	2 08	—	—	0.00357[g]	0 95	0.00139	1 17
Halothane	224[b]	390	0 0087[c]	1.95	0 0074[d]	1 66	0.00645[g]	1 45	0 00410	1 60
Enflurane	96 5[a]	151	0 0267[a]	2.58	0.0168[d]	1 62	0 0123[g]	1.19	0 00506	0 76
Isoflurane	90 8[a]	150	0 0141[a]	1 28	0 0115[d]	1 04	0 00663[g]	0 60	0 00385	0 58
Diethyl ether	65[b]	95	0 0304[c]	1 98	0 0192[d]	1.25	0 032[d]	2 08	0 0168	1 60
Mean ± SE				2 01 ± 0 17		1 42 ± 0 11		1 41 ± 0 26		1 37 ± 0 28

[a]Ref. 71, [b]ref 72, [c]ref 73, [d]ref 74, [e]ref 75, [f]ref 76; [g]ref 77.
Values of oil/gas partition coefficient at 22°C taken from ref 58
ED_{50} values for *Drosophila* at 22°C taken from ref 78
*Righting reflex
Note: Flies are grown at 22°C and tested for anaesthetic response at the same temperature.

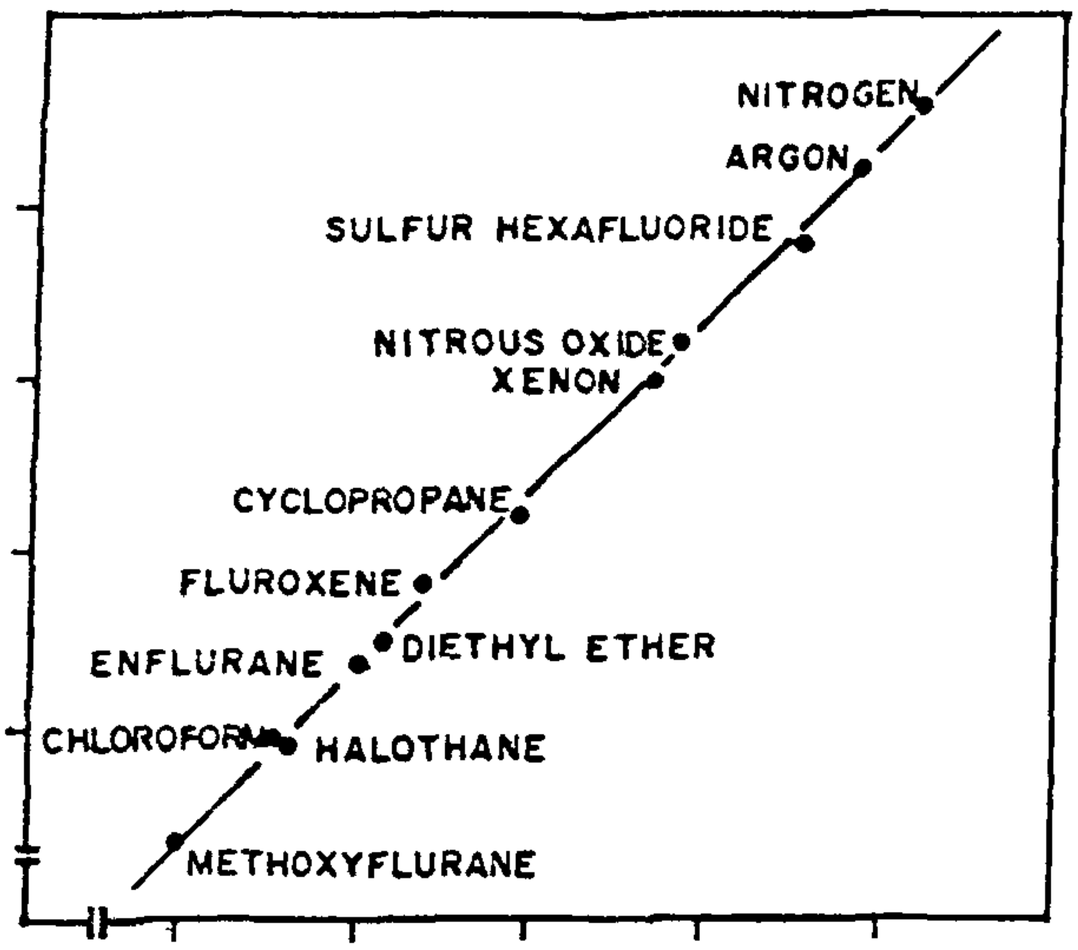


Figure 2. The correlation of anaesthetic potency with olive oil/gas partition coefficient (adapted from ref. 79). MAC refers to minimum alveolar concentration and is defined as that concentration of anaesthetic at 1 atm which induces anaesthesia in 50% of the subjects.

Where do anaesthetics act?

It has never been questioned that the volatile general anaesthetics have their primary target on neuronal membranes. This also fits in with the Meyer–Overton rule. The stereospecific action of certain anaesthetics[15–17] is suggestive of proteins, either directly or indirectly, as targets of anaesthetic action. However, these results show either no differential effect or differences that are so small that it is impossible to infer the nature of the target.

It has been shown by a variety of biophysical measurements that anaesthetics increase the fluidity of membranes and lipid bilayers[18]. However, the observed increase in fluidity[5, 14] was at relatively higher concentrations of anaesthetics. Later studies[5, 14, 19] conducted at clinically relevant concentrations of anaesthetics showed that the changes in lipid bilayer structure and fluidity are virtually undetectable. Earlier it was also shown[20, 21] that clinical concentrations of general anaesthetics produce sizeable changes in the surface pressure of lipid monolayers held at a constant area at an air/water interface. However, X-ray and neutron diffraction studies[5, 14] could not detect any significant change in the structure or thickness of lipid bilayers exposed to surgical concentrations of anaesthetics. Other lipid parameters like phase transition and permeability[22, 23] have also been shown to be affected by general anaesthetics but the changes in these parameters are not so drastic[22, 24–26]. These results are understandable in the light of the amphipathic nature of lipids themselves and have been hardly enlightening. It is conceivable that lipids in membranes are in heterogeneous patches – specific membrane proteins surrounded by specific lipids with more or less affinity for anaesthetics. Proteins, on the other hand, have a high degree of specificity of action. However, anaesthetic literature is replete with results that indicate interaction of anaesthetics with almost every macromolecule involved in neuronal function. This leaves very little room for a cohesive model as to how anaesthetics actually depress neuronal function.

Anaesthetics bind to different classes of proteins, e.g. bovine serum albumin (BSA), haemoglobin, lactoglobulin, adenylate kinase and protein kinase C[27–32] but one protein, the light-emitting Luciferase enzyme, appears to be different as far as sensitivity to anaesthetics is concerned. Anaesthetic agents not only inhibit the activity of this enzyme at clinically relevant concentrations[33] but the inhibition appears to be competitive in nature and in some cases pressure reversal has also been shown. Franks and Lieb[34, 35] have shown that the potency of anaesthetics correlated well with their ability to inhibit the activity of firefly Luciferase in addition to correlation with olive oil/water partition coefficient. How does a protein with fixed molecular dimensions and specificity for a particular substrate molecule, luciferin, accomodate molecular species as diverse as anaesthetics? Some workers have suggested that the site may accomodate a few of smaller-size molecules compared to say only one of the larger-size molecule. But the scenario does not look quite so simple.

It is conceivable that the anaesthetic molecules possess properties not related to anaesthesia *per se* that help them to reach the actual site before interacting with it specifically and causing anaesthesia. These properties are reflected by the Meyer–Overton rule and the cut-off effect. The exact mechanism by which anaesthesia results is still an open question. It is not unlikely that several specific effects seen with the functions of neuronal macromolecules are incidental to anaesthetic partitioning into neuronal membranes and not causative of anaesthesia. On the other hand, one cannot rule out the possibility that the opening of a potassium channel, closing of a sodium channel or blockage of synaptic transmission is the primary effect. The wide variety of suggested targets leaves room for questions as to what molecular targets anaesthetics act on; or which regions of the brain primarily produce the state of unconsciousness as seen in an anaesthetized animal? It is in this context that a genetic approach becomes highly relevant.

The genetic approach

Complex physiological phenomena like learning, memory, alcoholism and anaesthesia are effectively seen at the level of the whole animal. These studies therefore require a systems approach and analysis of single cells

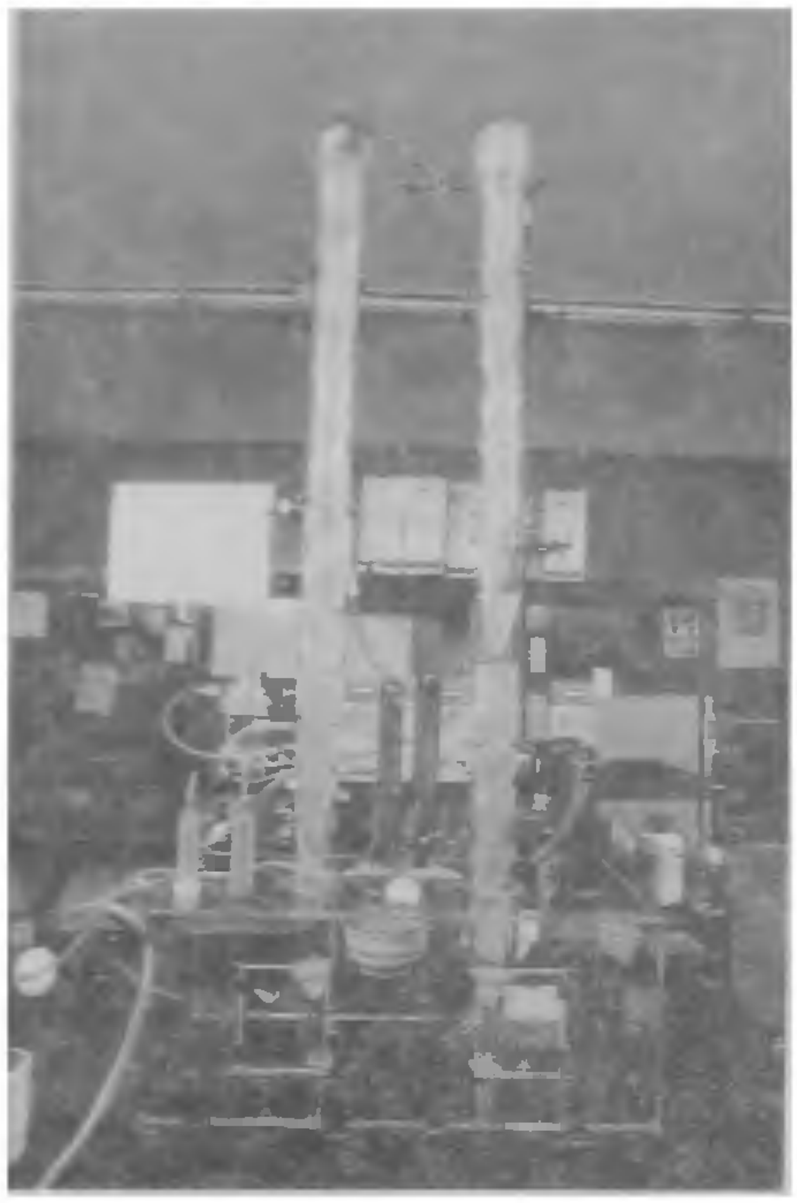
is not likely to provide an understanding of the behavioural changes. However, approaches like electrophysiology, simple model systems and genetics have yielded important information regarding the process of learning and memory[36]. Our contention is that a genetic approach supplemented by cellular physiology would yield better insight into the anaesthetized state.

Critical cellular components that may be involved in the response to inhalational anaesthetics are also the targets of mutational changes. Such changes that result in a consistent pattern of altered response to anaesthetics are what we seek in a genetic approach. These mutations will be of value in identifying specific molecular targets of anaesthetics if they exist or macromolecules in the pathway of anaesthetic action. Such molecules could be manipulated to change the time and site of their action to yield valuable clues as to how anaesthetics act. Such a manipulation needs simple model systems in which the response can be quantified and the genetics is facile. Two model systems *C. elegans* and *D. melanogaster* show great promise in this respect. Mutations that cause the organisms to deviate from the Meyer–Overton relationship have been identified[37–45, 65] in both these systems. Such mutations may have altered the site of anaesthetic action. Once mutations that alter sensitivity to anaesthetics are obtained, it is possible to look for second site mutations that affect the robustness of these phenotypes. Such 'enhancers' and 'suppressors' reflect interactions of the gene products[46, 47] at molecular level, either directly or as part of a cascade of physiological change. These will be of value in understanding the 'molecular cast' of the anaesthetic response. Genetics also offers the powerful tool of 'mosaic analysis'[48–51] where one can create patches of mutant tissues in an otherwise wild-type background and *vice versa*. Correlation of anaesthetic response with a particular mosaic patch in an otherwise wild-type background could yield valuable clues to the focus of anaesthetic action in the nervous system. For example, if a chimeric organism with only central nervous system, fully or partly mutated, shows an altered response (resistance or sensitivity) to anaesthetics, it will clearly indicate that the site of this mutant patch is the focus of anaesthesia. In fact, our experiments with *har38* (unpublished results) clearly indicate that only a part of the central nervous system (the cephalic ganglion) is required for the response to general anaesthetics. We are now trying to map the site even more finely. By creating different alleles of the same mutated locus, which is possible and routinely done in *Drosophila*, the function of different domains of a protein *vis-à-vis* anaesthesia may also be worked out. Genetics thus offers the study of anaesthetic action at the level of whole organism (by mosaic analysis) and also at the level of single putative target molecule (by creating allelic series of mutations).

Flies, like humans, can be rendered unconscious by knock-out insults like mechanical shock, temperature changes as also with anesthetics. This state of unconsciousness which renders the organism immobile invariably involves neural elements and at the same time is reversible without severe permanent loss of function. Study of such states is important for understanding the functioning of the nervous system. In files, for example, it has been possible to get important information on nervous system by studying temperature-induced paralytics like *shibire*[52], *para*[53], *comatose*[52], *nap*(ts)[54], *stoned*[55], etc. Similarly mutants which are easily knocked out, like *bang sensitive*[55], *bang senseless*[56], *easily shocked*[57], have also been of value.

Dissection of the molecular basis of the anaesthetic state by genetic means was inconceivable until very recently. The first systematic search for point mutations that alter response to volatile anaesthetics was initiated in the laboratory of Howard Nash[43]. The screen was facilitated by the study of the behaviour of flies in a column (inebriometer[43]; Figure 3) equilibrated with anaesthetic concentrations of halothane. Four mutations, which we now know fall in three complementation groups, were identified. These mutants, initially isolated as resistant to halothane, later proved to be resistant to some other anaesthetics as well[58] (Table 2). Of the four mutants isolated, *har38* and *har85* are recessive, *har56* is semidominant whereas *Har63* is dominant. They also differ in their anaesthetic response; *har38* is more resistant, followed by *har85* and *Har63* whereas *har56* is the least resistant (based on population behaviour). It is possible that mutants isolated as resistant to anaesthetics could be altered primarily in the metabolism of these compounds; such pharmacokinetic mutants may have little value in the understanding of the neural response to anaesthetics. However, two of these mutants, *har38* and *har85*, which we now know are allelic (unpublished results), display abnormal behaviour in the absence of anaesthetics. The most obvious phenotype is that these mutants have low viability and the abdomens of adult flies are more slender and elongated than wild-type flies. Most interestingly, the flies perform poorly in a standard counter-current geotaxis assay. This is largely because they tend to walk fits and starts, taking a few steps and then stopping before starting again. We have now characterized these mutants thoroughly on the basis of this abnormal walking behaviour and it correlates well with their anaesthetic resistance (unpublished results). All the above three traits, i.e., low viability, abnormal walking behaviour and abdominal morphology, map to the same deficiencies that uncover the anaesthetic-resistant behaviour. So, it is clear that the phenotypes of *har38* and *har85* do not depend solely on pharmacological challenge. These flies have suffered alterations in physiology,

a



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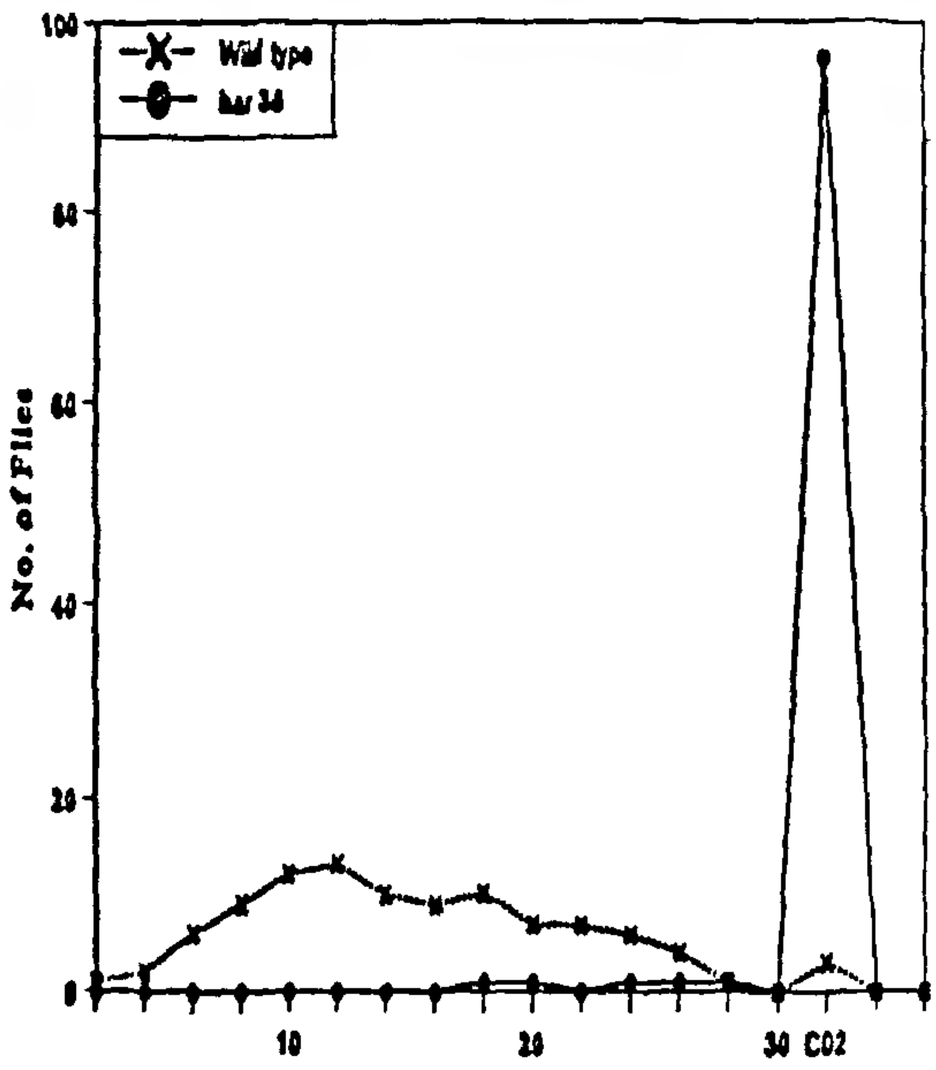

Figure 3 *a*, The 'inebriometer' used for fractionating *Drosophila* resistant to anaesthetics. *b*, The elution pattern of flies from the inebriometer. Flies were grown at 22°C and collected when they were 24–36 h old. The column was equilibrated with a particular concentration of anaesthetic for 15 min and after that flies, to be checked for resistance, were loaded at the top. Fractions were collected after every 2 min. Flies which lose postural control first are eluted first. After 30 min, anaesthetic is stopped and the resistant flies are eluted out with a mild puff of carbon dioxide.

Table 2. Anaesthetic resistance and *har* mutants[43, 58] and map position of *har* mutants

Anaesthetic	*har38/85*	*Har63*	*har56*
Chloroform	+	–	–
Trichloroethylene	+	–	+
Methoxyflurane	+	+	+
Diethyl ether	–	–	–
Enflurane	–	+	+
Isoflurane	–	±	–
Halothane	+	+	+

+ Denotes resistance; – denotes sensitivity.

har38/85	12E2–12E5
Har63	between ct and v[42]
har56	between v and g[42]

(Mir and Krishnan, unpublished).

presumably reflecting changes in the neuromuscular system that render them atypical in their response to anaesthetics.

It is worthwhile mentioning here that a variant (*Eth*) obtained several years ago by Japanese workers[38] showed resistance to ether (assayed as mortality rate after 24 h of exposure to ether). However, the genetics of this phenotype was intractable[38]. Yet another mutation, *91R*, which was originally isolated as resistant to DDT[59], turned out to be resistant to halothane (Dapkus *et al.*, personal communication). Tinkelberg *et al.*[60] observed that *shaker* alleles behave differentially towards anaesthetic isoflurane. They showed that Sh^{null} and Sh^{ks133} are resistant in the tail flick assay (ability of fly to move away from a hot spot). However, in the inebriometer *Shaker* alleles particularly Sh^{Ks133} is hypersensitive to halothane[45], and Sh^{5} alone is resistant to isoflurane and enflurane (Krishnan, unpublished observation). In parallel to the developments in the fly, mutations in the *C. elegans* which render the worm sensitive to various anaesthetics have been identified. *unc-79* and *unc-80* make the worm more sensitive to halothane, whereas *unc-9* suppresses both these mutations[61]. However, it has not been possible to obtain anaesthetic-resistant mutations in *C. elegans*. The worm needs long exposures (~ 2 h) to get immobilized and the concentrations needed are very high. *D. melanogaster* has some advantages over the nematode *C. elegans* in the study of the anaesthetic response. More realistic concentrations of anaesthetics are used (Table 1) and the onset of anaesthesia is brief. The interesting feature of the anaesthetic mutants in both the model systems is that these mutants do not show the same response towards all the anaesthetics[58, 61–65] and even discriminate between isomers of the same compound[58 66]. These studies argue against the nonspecific action of anaesthetics and explore the possibility of multiple targets[58 62].

Genetics as a tool to address the problem of identifying molecular targets of anaesthetics, is very recent as compared

to the history of anaesthesia (summarized below).

History of anaesthesia

Year	Event
1776	Priestly synthesized nitrous oxide but its anaesthetic properties became known 20 years later.
1816	Michael Faraday discovered the anaesthetic properties of diethyl ether.
1846	First surgery done under ether.
1847	James Simpson introduced chloroform in clinical practice.
1929	Anaesthetic properties of cyclopropane were discovered.
1956	Halothane introduced in clinical practice.

Only a handful of mutants are available (summarized below) and hardly anything is known at the molecular level.

Genetic studies of anaesthesia in *Drosophila*

Year	Event
1955	Rasmuson[37] isolated ether-sensitive mutant.
1967	Ogaki *et al.*[38] showed inheritance of ether resistance.
1972	Gamo *et al.* showed inheritance of chloroform resistance.
1990	Krishnan and Nash[42] isolated halothane-resistant mutants.
1991	Tinklenberg *et al.* studied the effect of anaesthetics on *Shaker* mutants.
1993	Campbell and Nash[58] studied the effect of anaesthetics on various *har* mutants.
1995	Leibovitch *et al.*[45] studied the response of various channel mutants to different anaesthetics.

Molecular cloning of the genes will be a fruitful approach in simple model systems like *Drosophila* and this could be extended, by looking for homologues, to higher systems.

Conclusion

Many fundamental observations are compatible with the idea that anaesthetics partition into neuronal membranes. As of now, it is unclear as to whether there are specificities with respect to the various regions of the brain and whether there are specific macromolecules on the cell surface that are primary targets. It is equally likely that it can be either a single region and a single target molecule or several regions and multiple target molecules. A whole lot of compounds are excluded from the list of anaesthetics, probably due to their inability to reach appropriate targets because of the limitations of barriers. The drawback of current strategies is that they lack a clear approach to identify the cellular elements of the anaesthetic process. While the importance of the genetic approach to this problem cannot be overstated, it promises to be different from other approaches since here we study the whole animal, avoiding most of the artefacts inherent in other approaches. Certainly, this approach will provide a serious input into identification of the cellular elements involved in the process of anaesthesia.

Some observations related to anaesthesia

Malignant hyperthermia (MH)

'It is a covert pharmacogenetic myopathy which incommodes the sufferer little, if at all, in the normal course of events but, in response to exposure to general anaesthesia, manifests in the susceptible individual as a life-threatening syndrome of muscle hypercatabolism and rigor, accompanied by a rapid and inexorably progressive rise in body temperature to extremes of the order of 42°C. Suxamethonium (succinyl choline) and halothane are the two most potent – almost specific – agents for triggering MH.

The possibility that strains of certain breeds of pigs might serve as a valid animal model of MH was suggested by the observation, in litter mate pigs anaesthetized with halothane, of muscle rigor and hyperthermia in response to the injection of suxamethonium reported by Hall and coworkers (1966); thereafter, Harison and coworkers (1968) reported the same reaction but in response to administration of halothane alone. This MH response in pigs, later identified with the porcine stress syndrome, was shown to be heritable, confined to selected strains of a few breeds (Landrace, Pietrain, Poland, China) and to mirror the human reaction in every way' (ref. 67).

Malignant diagnosis

'The last thing one needs during an operation is a serious reaction to general anaesthetics, but for sufferers of the rare disorder malignant hyperthermia (MH), the resulting sudden paralysis can be fatal if unchecked. Linkage studies have incriminated the rynodine receptor (*RYR1*), which controls release of calcium ions in muscle cells, but a putative mutation has only just been identified. E. F. Gillard *et al.* (*Genomics*, 1991, **11**, 751–755) describes a point mutation in the *PYR1* gene in affected members of one out of 35 MH families, which substitutes an arginine residue for cysteine. The analogous mutation is found in five strains of pigs that suffer the related porcine stress syndrome. However, the molecular basis of most MH cases remains to be determined and may not be confined to the PYR gene' (ref. 68).

Hypersensitivity to carbon dioxide

L'Hentier and Trissier[69] discovered a form of carbon dioxide hypersensitivity in *Drosophila* that showed an unusual pattern of inheritance. Later on, this phenomenon was linked to the presence of a symbiont (virus) in hypersensitive flies.

1. Budavari, S., *The Merck Index*, 11th edn, Merck & Co., Rahway, NJ, 1989
2. Meyer, H., *Arch. Exp Path. Pharmak.* (*Naunyn-Schmiedebergs*), 1899, **42**, 109–118.
3. Meyer, H., *Arch. Exp. Path. Pharmak.* (*Naunyn-Schmiedebergs*), 1901, **46**, 338–346.
4. Overton, E., *Studien uber die Narkose* (ed. Fischer, G.), 1901.
5. Franks, N. P. and Lieb, W. R., *Nature*, 1978, **274**, 339–342.
6. Katz, Y. and Simon, S. A., *Biochim. Biophys. Acta*, 1977, **471**, 1–15.
7. Richards, C. D., in *Tropical Reviews in Anaesthesia* (eds Norman, J. and Whitwam, J. G.), Wright, Bristol, 1980, vol. 1, pp. 1–84
8. Miller, K. W. and Smith, E. B., in *A Guide to Molecular Pharmacology – Toxicology* (ed. Featherstone, R. M.), Dekker, New York, 1973, vol. 1, pp. 427–475
9. Pauling, L., *Science*, 1961, **134**, 15–21.
10. Mullins, L. J., *Chem. Rev.*, 1954, **54**, 289–323.
11. Pringle, M. J., Brown, K. B. and Miller, K. W., *Mol. Pharmacol.*, 1981, **19**, 49–55.
12. Lever, M. J., Miller, K. W., Paton, W. D. and Smith, E. B., *Nature*, 1971, **231**, 368–371.
13. Halsey, M. J. and Wardley-Smith, B., *Nature*, 1975, **257**, 811–813.
14. Franks, N. P. and Lieb, W. R., *J. Mol. Biol.*, 1979, **133**, 469–500.
15. Andrews, P. R. and Mark, L. C., *Anaesthesiology*, 1982, **57**, 314–320.
16. Harris, B., Moody, E. and Skolnick, P., *Eur. J. Pharmacol.*, 1992, **217**, 215–216.
17. Franks, N. P. and Lieb, W. R., *Science*, 1991, **254**, 427–430.
18. Metcalfe, J. C., Seeman, P. and Burgen, A. S. V., *Mol Pharmacol.*, 1968, **4**, 87–95.
19. Lieb, W. R., Koyalycsik, M. and Mendelsohn, R., *Biochim. Biophys. Acta*, 1982, **688**, 388–389.
20. Skou, J. C., *Biochim. Biophys. Acta*, 1958, **30**, 625–629.
21. Bangham, A. D., Standish, M. M. and Miller, N., *Nature*, 1965, **208**, 1295–1297.
22. Hill, M. W., *Biochim. Biophys Acta*, 1974, **356**, 117–124.
23. Johnson, S. M., Miller, K. W. and Bangham, A. D., *Biochim. Biophys Acta*, 1973, **307**, 42–57.
24. Mountcastle, D. B., Biltonen, R. L. and Halsey, M. J., *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4906–4910.
25. Kamaya, H., Ueda, I., Moore, P. S. and Eyring, H., *Biochim. Biophys. Acta*, 1979, **550**, 131–137.
26. Johnson, S. M. and Bangham, A. D., *Biochim. Biophys. Acta*, 1969, **193**, 92–104.
27. Wishnia, A. and Pinder, T., *Biochemistry*, 1964, **3**, 1377–1384.
28. Kiehs, K., Hansch, C. and Moore, L., *Biochemistry*, 1966, **5**, 2602–2605.
29. Wishnia, A., *Biochemistry*, 1969, **8**, 5064–5070.
30. Wishnia, A. and Pinder, T. W., *Biochemistry*, 1966, **5**, 1534–1542.
31. Sachsenheimer, W., Pai, E. F., Schultz, G. and Schirmer, R. H., *FEBS Lett.*, 1977, **79**, 310–312.
32. Slater, S. J., Cox, K. J. A., Lombardi, J. V., Ho, C., Kelly, M. B., Rubin, E. and Stubbs, C. D., *Nature*, 1993, **364**, 82–84.
33. Ueda, I. and Kamaya, H., *Anaesthesiology*, 1973, **38**, 425–436.
34. Franks, N. P. and Lieb, W. R., *Nature*, 1984, **310**, 599–601.
35. Franks, N. P. and Lieb, W. R., *Nature*, 1994, **367**, 607–614.
36. Griffith, L. C., Verselis, L. M., Aitken, K. M., Kyriacou, C. P., Danho, W. and Greenspan, R. J., *Neuron*, 1993, **10**, 501–509.
37. Rasmuson, B., *Hereditas*, 1955, **41**, 147–208.
38. Ogaki, M., Nakashima-Tanaka, E. and Murakami, S., *Jpn J. Genet.*, 1967, **42**, 387–394.
39. Deery, B. J. and Parsons, P. A., *Theor. Appl. Genet*, 1972, **42**, 208–214.
40. Gamo, S., Ogaki, M. and Nakashima-Tanaka, E., *Jpn. J. Genet.*, 1979, **54**, 229–234
41. Kirschfeld, K. and Baier-Rogowski, V., *Biol. Cybern.*, 1988, **58**, 1–11.
42. Krishnan, K. S. and Nash, H. A., *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 8632–8636.
43. Nash, H. A., Campbell, D. B. and Krishnan, K. S., *Ann. NY Acad. Sci.*, 1991, **625**, 540–544.
44. Tinklenberg, J. A., Segal, I. S., Guo, T. Z. and Maze, M., *Ann. NY Acad. Sci.*, 1991, **625**, 532–539.
45. Leibovitch, B. A., Campbell, D. B., Krishnan, K. S. and Nash, H. A., *J. Neurogenet.*, 1995, **10**, 1–13.
46. Ashburner, M., *Drosophila: A Laboratory Hand Book*, Cold Spring Harbour Laboratory Press, New York, 1989.
47. Lindsley, D. C. and Zimm, G. G., *The Genome of Drosophila melanogaster*, Academic Press, 1992.
48. Suzuki, D. T., Grigliatti, T. and Williamson, R., *Proc. Natl. Acad. Sci. USA*, 1971, **68**, 890–893.
49. Grigliatti, T., Suzuki, D. T. and Williamson, R., *Develop. Biol.* 1972, **28**, 352–371.
50. Golic, K. G. and Lindquist, S., *Cell*, 1989, **59**, 499–509.
51. Golic, K. G., *Science*, 1991, **252**, 958–961.
52. Siddiqi, O. and Benzer, S., *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 5253–5257.
53. Suzuki, D. T., Grigliatti, T. and Williamson, R., *Proc. Natl. Acad. Sci USA*, 1971, **68**, 890–893.
54. Wu, C. F., Ganetzky, B., Jan, L. Y., Jan, Y. N. and Benzer, S., *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4047–4051.
55. Grigliatti, T., Hall, L., Rosenbluth, R. and Suzuki, D. T., *Mol. Gen. Genet.*, 1973, **120**, 107–114
56. Jan, L. Y. and Jan, Y. N., *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 515–519.
57. Ganetzky, B. and Wu, C. F., *Genetics*, 1982, **100**, 597–614.
58. Campbell, D. B. and Nash, H. A., *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 2135.
59. Merrell, D. J. and Underhill, J. C., *J. Econ. Entomol.*, 1956, **49**, 300–306.
60. Tinkelberg, J. A., Segal, I. S., Tianzhi, G. and Maze, M., in *Molecular and Cellular Mechanisms of Alcohols and Anaesthetics* (*Ann. NY Acad. Sci.*), 1991, **625**, 532–539.
61. Sedensky, M. M. and Meneely, P. M., *Science*, 1987, **236**, 952–954.
62. Morgan, P. G., Sedensky, M. M. and Meneely, P. M., *Ann. NY Acad. Sci.*, 1991, **625**, 524–531.
63. Morgan, P. G. and Cascorbi, H. F., *Anaesthesiology*, 1985, **62**, 738–744.
64. Morgan, P. G., Sedensky, M. M., Meneely, P. M. and Cascorbi, H. F., *Anaesthesiology*, 1988, **69**, 246–251.
65. Morgan, P. G., Sedensky, M. M. and Meneely, P. M., *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 2965–2969.
66. Sedensky, M. M., Cascorbi, H. F., Meinwald, J., Radford, P. and Morgan, P. G., *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 10054–10058.
67. Nunn, J. F., Utting, J. E. and Brown, B. R., in *General Anaesthesia*, Butterworths, London, 1989, 5th edn, p. 655.
68. Lindley, D., *Nature*, 1991, **354**, 22.
69. L'Heritier, P. H. and Trissier, G., *CR Acad. Sci.* (*Paris*), 1937, **205**, 1099–1101.
70. Miller, R. D., in *Anaesthesia*, 3rd edn, Churchill Livingstone, New York, 1990, vol. 1, p. 52.
71. Koblin, D. D., Eger, E. I., Johnson, B. H., Collins, P., Harper, M. M., Terrell, R. C. and Speers, L., *Anaesthesiology*, 1981, **54**, 314.
72. Kent, D. W., Halssey, M. J. and Eger, E. I., *Anesth. Analg.*, 1977, **56**, 97.

73 Eger, E I , Brandstater, B and Saidman, L J , *Anaesthesiology*, 1965, **26**, 771
74 Quasha, A, L , Eger, E. I and Tinker, J H , *Anaesthesiology*, 1980, **53**, 315
75 Koblin, D D , Deady, J. E. And Eger, E I , *Anaesthesiology*, 1982, **56**, 18
76 Eger, E I , Lundgren, C , Miller, S. L. and Stevens, W C , *Anaesthesiology*, 1969, **30**, 129
77 Deady, J E , Koblin, D D and Eger, E. I , *Anesth. Analog* , 1981, **60**, 380
78 Allada, R and Nash, H. A., *Anesth Analg* , 1993, **77**, 19–26
79 Gillman, A G , Rall, T. W , Nies, A S and Taylor, P , in *The Pharmacological Basis of Therapeutics*, 8th edn, Pergamon, New York, 1991, vol 1, p. 282

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Magnesium deficiency and the cardiovascular system

K. Shivakumar

Division of Cellular and Molecular Cardiology, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum 695 011, India

Magnesium plays an important role in maintaining the structural and functional integrity of the cardiovascular system. Its influence on cardiac ion channels has immense clinical implications and is the subject of several incisive investigations. Magnesium deficiency may lead to a wide spectrum of vascular and cardiac complications. This article reviews evidence that magnesium deficiency promotes hypercoagulability of blood, atherogenesis, vasoconstriction, cardiac arrhythmias and cardiac muscle damage. Mechanisms underlying these effects are briefly discussed. Further, it is proposed that the myocardial lesions of chronic magnesium deficiency may result from recurrent episodes of mild ischaemia and reperfusion and consequent free-radical generation.

ONE of the most abundant cations within mammalian cells, magnesium (Mg) is an important metabolic cofactor, particularly in transphosphorylation reactions. It has been known for a long time that more than 300 enzymatic reactions require Mg and that the biosynthetic repertoire of the cell is critically dependent on it. Mg also functions as a transmembrane and intracellular modulator of other ions. However, it was only recently that the regulatory role of the element has been recognized in the wake of the discovery that intracellular free Mg, $[Mg^{2+}]_i$, is in the submillimolar range and that several intracellular systems have K_m values for Mg within this range[1]. This opens the possibility that $[Mg^{2+}]_i$ may vary physiologically and act as a physiological modulator.

The emergence of Mg as a premier cardiovascular cation follows important observations on the cardiovascular consequences of Mg deficiency which is no longer considered a mere laboratory phenomenon. Understandably, there is increasing interest in the cellular and molecular actions of Mg and their relevance to clinically recognized cardiovascular events in humans. This review discusses briefly the link between Mg deficiency and abnormalities in cardiovascular function and examines the underlying mechanisms.

Incidence of magnesium deficiency

A major problem in assessing the role of Mg status in the aetiology of diseases is the inadequacy of the indicators of Mg status[2]. As only 1% of the total body Mg is in the extracellular fluid, the total serum Mg may not reflect body stores. It is now amply clear that Mg depletion in tissues can exist despite normal serum Mg levels[2]. Cardiac disorders have been described[2] in cases with normal serum Mg and lower tissue Mg. Low serum Mg and normal cellular total Mg content without clinical signs of Mg deficiency have also been reported. Further, the reported normal values for serum Mg fall within a rather broad range, making it hard to know what constitutes an acceptable level. Since <10% of intracellular free Mg is freely exchangeable or ionizable Mg^{2+}, it is suggested that this fraction could be a better determinant of potential consequences of Mg deficiency than either serum Mg or total cellular Mg^{2+}. Nuclear magnetic resonance promises a reliable correlation between $[Mg]_i$ and diagnostic indices of Mg deficiency, but is not easily accessible[2].

Be that as it may, a series of clinical reports in the early 1960s helped focus attention on the occurrence of