

PHARMACOLOGICAL PROPERTIES RELATED TO THE MECHANISM OF ACTION OF POTENT ANALGESIC DRUGS

CHARLES A. WINTER

*Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486, U.S.A.
and Woman's Medical College of Pennsylvania,
Philadelphia, Pennsylvania 19129, U.S.A.*

Revolutionary changes in many types of drug therapy have become rather commonplace within the past generation, but they have been relatively slow to appear in the field of analgesia. As recently as the mid-1950s, it was a widely held view that a compound could have potent analgesic activity only if it conformed to certain structural configurations.

Braenden *et al.*⁷ listed these structural requirements as follows: (a) a tertiary nitrogen to which a small alkyl group is attached; (b) a central carbon with none of its valences connected to a hydrogen atom; (c) a phenyl group (or isostere) connected to the central carbon; and (d) a two-carbon chain between the central carbon and the tertiary nitrogen.

Many active compounds, some of them much more potent than morphine, were synthesized on the basis of this structural design. All of them had certain biological properties in common, including: (a) activity in animal tests utilizing the reaction of the organism to a thermal stimulus; (b) development of tolerance; and (c) addiction liability.

The animal tests which used thermal stimuli for analgesic testing were based on the work of Hardy, Wolff and Goodell²⁴. They included the mouse hot plate technique of Woolfe and Macdonald⁶⁵ and the rat tail-flick test of D'Amour and Smith¹². Other tests used pressure stimuli instead of heat⁶³.

The compounds whose activity was discovered by these testing procedures were so consistent in their biological properties that in 1956 Schaumann expressed the view, held also by many other workers, when he said: 'It will therefore not be possible to find morphine-like analgesics without addiction liability'⁴⁹.

Progress in analgesic research has markedly accelerated since it has become clear that the views expressed such a short time ago are no longer tenable. Some of the evidence which altered the opinions formerly widely accepted regarding structural requirements has been mentioned in reviews by Archer and Harris¹, Hardy and Howell²⁵ and Brossi *et al.*⁸.

The compounds shown in *Figure 1*, all potent analgesics, illustrate some of these principles. Compound (I), anileridine⁴⁵ was one of the first to demonstrate that the substituent on the tertiary nitrogen need not be small. Compound (II), diampromide, shows that a tertiary nitrogen can substitute for the central carbon. Compound (III) is a benzimidazole derivative about 1000 times the potency of morphine but it possesses no quaternary carbon.

The 4th compound, methotrimeprazine, is remarkable in that clinical reports show it to be a potent analgesic agent, though it does not resemble morphine either chemically or pharmacologically. The pharmacological actions resemble those of chlorpromazine, according to Courvoisier¹⁰. Analgesic activity could be detected in animal tests^{11, 38}, but the significance of the results was somewhat doubtful because of the sedative properties of the compound. In man, there is no doubt that methotrimeprazine is a potent analgesic agent quite apart from its sedative action. This has been confirmed

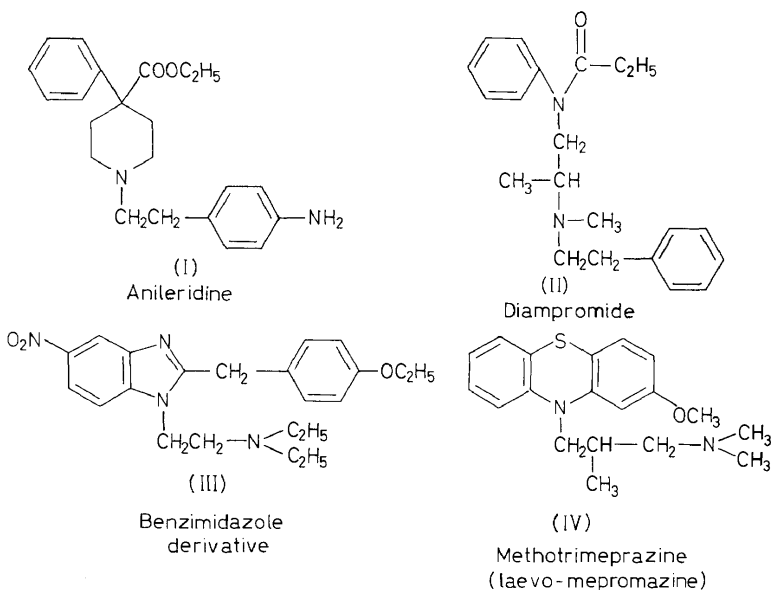
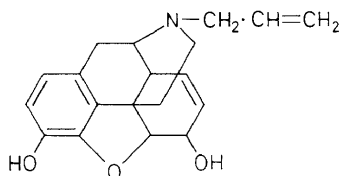


Figure 1. Potent analgesic compounds invalidating former views of essential structural requirements.

in patients with pain due to cancer² as well as in post-operative and labour pains³⁶. Methotrimeprazine depresses respiration less than does an equi-analgesic dose of morphine⁴⁶ and is apparently free of morphine-like addiction properties¹⁹. Its usefulness may be limited by the high incidence of side effects, especially in ambulatory patients. The most prominent of these are sedation, pain at the injection site, and orthostatic hypotension.

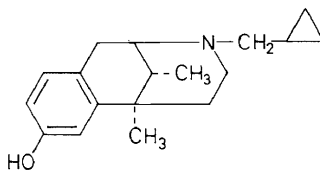
A development that has attracted a great deal of attention is the discovery that morphine antagonists have potent analgesic activity in man. Orahovats *et al.*⁴⁴ found that by mixing nalorphine with morphine in appropriate concentrations, analgesia (as measured by responses to thermal stimuli in animals) could be retained while the narcotic side effects of morphine were substantially reduced. In attempting to obtain similar results in man, Lasagna and Beecher³⁵ in 1954 could find no advantage of mixtures, but discovered that nalorphine is an effective analgesic agent. This finding was soon confirmed by others, but nalorphine proved to be impractical for therapeutic purposes because of psychic side effects.

Keats and Telford³² characterized nalorphine as the 'long-sought potent non-addicting analgesic'. Instead of substituting for morphine in the physically dependent addict, nalorphine provokes the abstinence syndrome if the addict is currently on narcotic drugs, or intensifies the syndrome if opiate has recently been withdrawn. Nalorphine, therefore, is a diagnostic aid in opiate addiction as well as an antidote for opiate poisoning, but it produces such bizarre psychic effects that it has been classed as a psychotomimetic agent. Fraser, in 1957, reviewed the human pharmacology of this compound¹⁷.



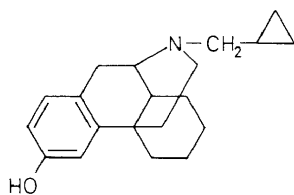
(V)

Nalorphine



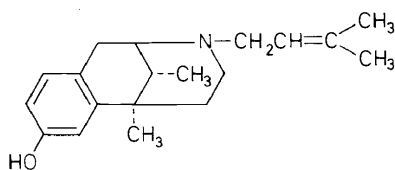
(VII)

Cyclazocine



(VI)

Cyclorphan



(VIII)

Pentazocine

Figure 2. Examples of opiate antagonists with potent analgesic activity.

Because of the great importance of these results, Keats undertook the clinical study of a number of compounds in the hope of finding an analgesic with the favourable properties of nalorphine, but without the psychotomimetic effects. The first efforts were disappointing⁵⁶ but rapid progress was made after the group at Sterling-Winthrop Research Institute, Rensselaer, New York, undertook to prepare and study narcotic antagonists in the benzomorphan series. Archer, Harris and their collaborators have discussed these investigations in a series of research and review publications^{1, 18, 26-28}.

The chemical structures of some of the opiate antagonists exhibiting potent analgesic activity are shown in *Figure 2*. Nalorphine, cyclorphan and cyclazocine are strongly antagonistic to morphine, while pentazocine is a relatively weak antagonist. The relative potency for morphine antagonism of these four compounds, as calculated from the data published by the Sterling-Winthrop group is as follows: nalorphine = 1, cyclorphan = 3.4, cyclazocine = 4.5, pentazocine = 0.01.

These four compounds differ considerably in potency as narcotic antagonists, but less widely as analgesic agents. The three strong antagonists share

the property of producing bizarre psychic effects in man, while pentazocine, the weak antagonist, rarely exhibits psychotomimetic activity except at high doses⁵⁹. Such data led Keats and Telford³⁴ to postulate a correlation between psychotomimetic activity and potency among narcotic antagonists. Available data are insufficient to test the general validity of Keats' hypothesis.

Pentazocine is sufficiently free from adverse psychic effects that Keats and Telford entitled their first clinical paper on its pain relieving properties 'A narcotic antagonist analgesic without psychotomimetic effect'³⁵. The lack of addiction liability was established by Fraser and Rosenberg²⁰ in studies at the Addiction Research Center in Lexington, Kentucky. Several investigators have confirmed the parenteral analgesic activity of pentazocine in a variety of clinical conditions; reports on side effects vary somewhat, but with the exception of physical dependence, most of the side effects are qualitatively similar to those of morphine^{3, 5}. Little has been published on the oral activity of pentazocine, but evidence indicates fairly good oral activity^{31, 59}.

The discovery of the analgesic effectiveness of the narcotic antagonists was somewhat delayed because the compounds failed to show analgesic activity in the animal tests that have traditionally been used to determine such activity. Indeed, the Sterling-Winthrop investigators expressed the view that the screening methods commonly used, such as the D'Amour-Smith rat tail-flick method and the Woolfe-Macdonald mouse hot plate technique, have actually hindered the search for non-addicting strong analgesics. These screening procedures were thought to be predictive of addiction liability rather than of pain relieving potentialities. Hence, Fraser and Harris¹⁸ said 'A good laboratory procedure for predicting the analgesic effectiveness of the narcotic antagonists in man is not available'.

The statement by Fraser and Harris may be unduly pessimistic. Several authors have described positive results in animal tests with the narcotic antagonists, and in some cases the rank order of potency correlated fairly well with clinical data. Pentazocine was active in the "flinch-jump" procedure employed by Evans and Bergner¹⁴ only in rather high doses. Nalorphine was inactive in the operant conditioning situation of Weiss and Laties⁶¹, though pentazocine was active. There are, on the other hand, at least three reports in which the relative potencies of cyclazocine, morphine, nalorphine and pentazocine in animal tests correlated to a significant degree with clinical effectiveness.

Taber *et al.*⁵³, employing a "phenylquinone writhing" test in mice, obtained relative potencies surprisingly close to those reported in man. Collier *et al.*⁹ used a variant of the technique, injecting acetylcholine to produce abdominal constrictions. *Table 1* shows the relative activities of the four compounds as described in these reports, compared to data from human trials. The correlation of animal data with clinical results in man is seldom if ever perfect, and the data obtained in these experiments correlate about as well as most.

The principal defect of the abdominal constriction method of analgesic testing is its lack of specificity, which leads to a large number of 'false positives'—that is, non-analgesic compounds which affect the response. Collier⁹ proposed various secondary tests to identify such compounds.

Winter and Flataker⁶⁴ described a modification of the well-known Randall-Selitto⁴⁷ procedure which was sensitive to the narcotic antagonists in rather low doses; a significant effect was obtained with nalorphine or cyclazocine at 0.22 mg/kg, or with pentazocine at 0.67 mg/kg. Our results, as well as those of Taber *et al.*⁵³ indicated a flatter slope for the antagonists than for morphine, a finding that accords with several clinical reports. Collier's data in mice, though not showing differences in slope among the compounds, exhibited a remarkably high rank correlation with human clinical potency ($\rho = 0.908$) among 14 parenterally administered analgesic drugs, and nearly as good a correlation ($\rho = 0.821$) among 20 orally

Table 1. Inhibition of the abdominal constriction response ('writhing') in mice by morphine and narcotic antagonists

Compound	Taber <i>et al.</i> ⁵³		Collier <i>et al.</i> ⁹		Relative potency in man†
	ED ₅₀ (mg/kg)	Relative potency	ED ₅₀ (mg/kg)	Relative potency	
Morphine	0.45	1	0.5	1	1
Cyclazocine	0.10	4.5	0.08	6.25	27.5
Pentazocine	2.00	0.22	2.74	0.2	0.4
Nalorphine	0.92	0.5	1.96	0.26	0.8

† For references, see Taber *et al.*⁵³

administered agents, including the antipyretic type of analgesics as well as the potent narcotics and narcotic antagonists. Our data, though demonstrating activity for the antagonists, did not seem to be well adapted for quantitative comparisons.

The findings with the narcotic antagonists have stimulated renewed activity in the search for non-addicting analgesics. Blumberg *et al.*⁶ recently announced another narcotic antagonist, the *N*-dimethylallyl derivative of oxymorphone, to be active in the mouse abdominal constriction test. It is a fairly potent narcotic antagonist, and results in man will be awaited with some interest in view of the psychotomimetic action of other potent antagonists.

Entirely new chemical structures are represented by the recently announced compounds shown in *Figure 3*. It is too early to tell the clinical usefulness of any of these compounds, but pharmacological studies^{23, 62} indicate that the first two, at least, have an interesting degree of analgesic activity.

Analgesic drugs are traditionally classified as either potent or mild. Potent analgesics are those which control severe pain, and include the narcotic analgesics, whether opium alkaloids or synthetic substances such as pethidine or methadone. It now seems clear that the concept of potent analgesics must be extended to include a variety of non-narcotic compounds, and the ability to relieve severe visceral pain need no longer be equated with narcotic or addicting properties. Thus, the field of analgesia is at last catching up with other aspects of medicine which have seen such revolutionary changes within the past couple of decades. It still seems necessary to retain a distinction between these compounds and the so-called 'mild' analgesics of the antipyretic type.

In addition to pain relieving properties, narcotic analgesics have other actions on the central nervous system. The bewildering variety of central nervous system effects which morphine displays has been studied a great deal, without shedding much light on the intimate mechanism of analgesic action. Most of the attention has been focused on events other than analgesia, generally on undesirable consequences of drug action. There is general agreement that morphine acts on the brain, but the compound is not preferentially distributed to the brain; in fact, brain levels are low compared

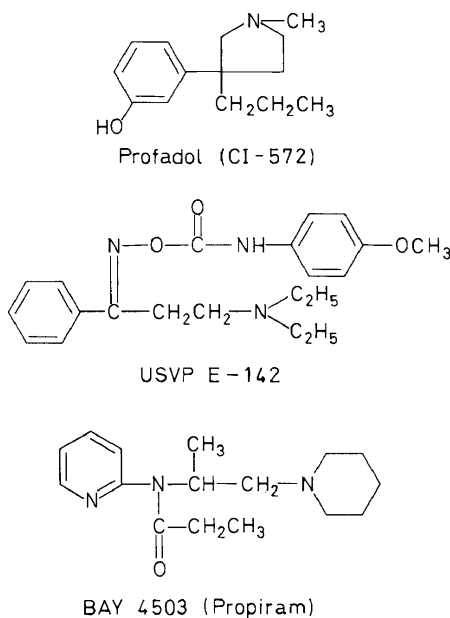


Figure 3. Some recently announced analgesic compounds.

to other tissues. The distribution within the brain appears to be the same as that of other bases, and it has been difficult to obtain evidence of any correlation between free morphine anywhere in the central nervous system and pharmacological effects of the compound.

The level of morphine in the brain associated with analgesia seems to be extremely small⁶⁰—probably of the order of a few nanograms per gram of tissue, but there is some evidence of a correlation between total brain levels and the level of analgesia. Such correlation was described for several potent analgesic agents by Miller and Elliott⁴⁰; Jóhannesson and Woods²⁹ found that when morphine was administered by different routes, different doses were required to effect a comparable degree of analgesia, but equianalgesic doses resulted in the same concentrations in the brain. In the presence of nalorphine no correlation was seen between the concentration of free morphine in the brain and pharmacological effect in non-tolerant dogs⁴¹, though nalorphine significantly decreased morphine concentration in tolerant dogs⁴². In all of these experiments, the distribution of morphine

in different portions of the brain was unrelated to any presumed 'pain pathway'.

Even less is known about the site of the analgesic action of the narcotic antagonists except that they are taken up by the brain^{9, 18}. There is evidence, however, that they compete with morphine for a receptor site, and that is presumably the basis for their morphine antagonistic action. It is tempting to speculate that the receptor site for analgesia may be the same for the antagonists as for morphine, but we lack direct evidence that this is so. Indeed, it seems rather curious that two compounds with nearly the same analgesic action in man, milligram for milligram, would oppose each other when administered together if the analgesic action of the antagonist were exerted at the same receptor site as that of the agonist.

Recent clinical studies of morphine-pentazocine combinations⁵⁹ indicate that pentazocine has a different action than nalorphine. Low doses of nalorphine antagonized the analgesic effect of morphine, while at high doses of nalorphine in combination with morphine the analgesia due only to the nalorphine was in evidence. Pentazocine, on the other hand, added to the analgesic effect of morphine throughout a wide range of doses. When subcellular preparations were made from the brains of cats treated with morphine or pentazocine, Ferrari and Connolly¹⁶ noted that the two drugs differed in their degree of binding to subcellular constituents of the cerebral cortex. The relation of this finding to analgesic receptors was not established.

A receptor site is often regarded as a complex configuration of chemical groups which react with a specific part of the drug molecule which fits the receptor like a key in a lock. A configuration for the 'analgesic receptor' has been postulated by Beckett⁴. The exact meaning of the term 'drug receptor' has been widely discussed recently, and it does not have the same connotation for all pharmacologists, as Fastier¹⁵ and Furchgott²¹ have pointed out. It is possible that the receptor is not a rigidly fixed, static structure, but it may be a site either in the cell or on its surface which interacts with a drug and which may result in changes in both molecules. If one accepts this more dynamic view of the receptor site, one can begin to see how compounds of very diverse structure may fit a common receptor and produce a common biological response. Fraser and Harris¹⁸ presented evidence that several potent analgesics and some 20 different antagonists may act on a common receptor site. The receptor sites for certain side effects are probably different from those involved in analgesia; hence, certain compounds may be potent analgesic agents but may differ somewhat in their other biological effects.

The consequences of drug-receptor interaction are biochemical or biophysical changes which form the basis of pharmacological action. Potent analgesic agents such as morphine induce many such changes but none of them has been identified with analgesia with certainty. Cholinesterase inhibition is a prominent property of some morphine-like compounds as well as of morphine antagonists. This action was at one time widely regarded as a possible biochemical basis for analgesia, but numerous studies have failed to establish a relationship between anticholinesterase activity and analgesia in a variety of chemical structures⁶³.

Biogenic amines, including epinephrine, norepinephrine, dopamine and 5-hydroxytryptamine, have been widely studied as potential mediators of analgesia. Several authors have reported analgesia produced by sympathomimetic amines, and analgesic compounds are said to affect brain levels of catecholamines⁶³. There is considerable disagreement among these reports, however, and there is likewise disagreement about the effect of depletion of brain amines upon the analgesic action of morphine.

Table 2 shows that morphine analgesia has variously been reported to be inhibited, unaffected or potentiated by pretreating the animals with

Table 2. Morphine analgesia as affected by reserpine

Species	Test	Reserpine dose and route (mg/kg)	Reserpine -Morphine time interval (h)	Morphine dose and route (mg/kg)	Reserpine effect on analgesia	Reference
Mice	Tail-flick	10 s.c.	2	10 s.c.	Inhibition	51
Mice	Tail-flick	2.5 s.c.	2 to 16	5 s.c.	Inhibition	52
Mice	Hot plate	2 i.p.	3	10 i.p.	Inhibition	39
Rats	Tail-flick	1 i.p.	3	4 i.p.	Inhibition	39
Mice	Haffner	1 s.c.	4	ED ₅₀ s.c. 5.6 → 29.0	Inhibition	50
Mice	Haffner	1 s.c.	1 to 90	20 i.p.	Inhibition	54
Mice	Writhing	5 s.c.	24	ED ₅₀ s.c. 0.9 → 2.5	Inhibition	48
Mice	Foot-flick	1 i.p.	4	10 i.p.	Inhibition	58
Rabbits	Tooth pulp	2 i.v.	24	5 i.v.	Inhibition	58
Rats	Hot plate	1-2 i.p.	4-5	1.25-5 s.c. 2.5-10 i.p.	No effect	29
Mice	Tail-flick	2.5 s.c.	2-4	5 s.c.	Potentiation	57
Mice	Hot plate	1-5 s.c.	2-48	1-5 s.c.	Potentiation	22

reserpine. The majority of authors thought that reserpine inhibited the effect of morphine, and cited this as evidence that morphine analgesia is mediated by norepinephrine, since reserpine is known to deplete brain norepinephrine.

The weakness of this argument is demonstrated by investigations of the effects of other amine depleting agents on morphine analgesia. Rudzik and Mennear⁴⁸ found that neither α -methyl-DOPA nor α -methyl-*m*-tyrosine affected morphine analgesia in mice although reserpine inhibited the effect of morphine. Since these animals were presumably depleted not only of catecholamines but of 5-hydroxytryptamine and dopamine as well, they suggested that the antagonism of morphine by reserpine may be due to some property of reserpine other than its ability to deplete catecholamines. Contrary results were obtained by Verri *et al.*⁵⁸ who reported that α -methyl-*m*-tyrosine pretreatment prevented morphine analgesia as effectively as did reserpine.

The dose of α -methyl-*m*-tyrosine used by the two groups of investigators was different, but the disagreement in their results cannot be entirely explained on that basis, since the positive result was obtained by the group using the lower dose. To complicate matters still further, a report by

Medakovic and Banic³⁹ states that pretreatment of mice with either reserpine or α -methyl-*m*-tyrosine inhibits morphine analgesia, while in rats the morphine effect is antagonized by reserpine but not by α -methyl-*m*-tyrosine.

It is obvious that the matter has not yet been settled. The observation of different responses in diverse species suggests the possibility that part of the conflicting results in mice might be accounted for by variability among genetic strains—for which mice are notorious. Whether this is the explanation for the discrepancies among results or not, any finding of full analgesic effect under conditions in which the biogenic amines have been depleted in any species should be convincing evidence that analgesia is not necessarily mediated by the amines concerned.

Still another possible mechanism to explain the action of potent analgesic agents is suggested by the work of the Japanese workers, Kakunaga *et al.*³⁹. They found that calcium ion injected in modest doses intracisternally or in larger doses subcutaneously or intraperitoneally markedly reduced the analgesic effectiveness of morphine. The inhibition was equally pronounced whether the Ca^{2+} was given with the morphine or after the morphine effect was fully established. Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), reversed the Ca^{2+} effect, or if given without Ca^{2+} they enhanced the action of morphine. The effect was specific, it was not shared by divalent ions other than calcium, it was not antagonized by potassium ions, and the calcium complexes of the chelating agents were ineffective. Some of these findings have been confirmed by Nutt⁴³ working in Keats' laboratory, who extended the observations to include the response of the guinea pig ileum to morphine.

The experiments above were undertaken because of the reports by Take-mori⁵⁵ and by Elliott *et al.*¹³ that morphine was able to inhibit potassium-stimulated respiration in brain slices *in vitro* and that this action of morphine was demonstrable only in conditions of calcium deficiency.

All of these observations are especially significant since it has long been known that Ca^{2+} is important for neural activity. Although the experimental facts do not elucidate the intimate mechanism of action of morphine, they suggest that a calcium-sensitive step is involved, and one is tempted to speculate that a membrane phenomenon at the cell surface may be involved, though if so, the participation of intracellular mechanisms in addition are not excluded. Attempted application of these findings to the pain relieving properties of the morphine antagonists has not appeared in the literature, to my knowledge. It will be awaited with interest. A recent report^{1a} that reserpine inhibits the rate of Ca^{2+} uptake by certain subcellular particles may be of some interest in view of the effects of both reserpine and Ca^{2+} on analgesia.

I cannot leave the subject of potent analgesic agents without mentioning some of the pitfalls involved in the interpretation of experimental findings with drugs possessing known biochemical effects. For example, if a compound which depletes brain catecholamines (such as reserpine) inhibits morphine analgesia, there is a tendency to say that the inhibition is a consequence of the amine depletion. It may be so, but one should always bear in mind the possibility of alternative explanations. On the basis of comparable experiments, others have thought that either 5-hydroxytryptamine or dopamine

is a mediator in the central action of analgesic agents. There are few drugs that have only one action, and it is well to consider that a given effect of a drug may be due to some biochemical or biophysical property quite different from the one we have focused attention on.

An analogous situation is that which involved monamine oxidase inhibitors (MAOI). It was once thought that antidepressant activity was the result of the inhibition of MAO, but we now know that there are potent and useful antidepressant drugs which do not inhibit MAO. Hence, regardless of the mechanism of action of MAOI, inhibition of MAO is not the equivalent of anti-depressant action. In a different field, anti-inflammatory compounds in general uncouple oxidative phosphorylation, and efforts have been made to equate the uncoupling with anti-inflammatory activity. These efforts have added much to our knowledge of some of the biological properties of various agents, but have not elucidated the mechanism of therapeutic efficacy. Similarly, I regard it as highly questionable that either brain catecholamines, 5-hydroxytryptamine, calcium ions, or any other single substance that has been studied will give the final elucidation of the way in which certain drugs relieve pain.

In this discussion, I have concentrated on the so-called 'potent' analgesics. It would take an equally long discussion to cover the 'mild' analgesics, including aspirin and phenacetin, or those which are primarily useful for pain relief in conditions such as spondylitis, osteoarthritis and acute gouty arthritis, encompassing such agents as phenylbutazone, indomethacin and the fenamates. Suffice it to say that a growing body of evidence indicates that the analgesic action of these compounds is mainly if not entirely peripheral.

Most of the evidence for a distinction between the central action of the more potent analgesics and the peripheral action of the antipyretic type has come from the laboratory of R. K. S. Lim³⁷. Collier *et al.*⁹ also commented that some of their results supported the idea that antipyretic drugs acted peripherally in their test, in contrast to the central actions of the narcotics and the narcotic antagonist analgesics.

The recent developments in the field of analgesia have made it clear that pain relief can be obtained without the risk of addiction. We may with some confidence look forward to the discovery of many more useful compounds, and the elucidation of their mode of action will constitute a challenge to the best efforts of pharmacologists and biochemists for many years to come.

References

- 1 S. Archer and L. S. Harris. *Progr. Drug Res.* **8**, 261 (1965).
- ^{1a} H. Balzar and M. Makinose. *Arch. exp. Path. u. Pharmak.* **259**, 151 (1968).
- ² W. T. Beaver, S. L. Wallenstein, R. W. Houde and A. Rogers. *Clin. Pharmacol. & Therap.* **7**, 436 (1966).
- ³ W. T. Beaver, S. L. Wallenstein, R. W. Houde and A. Rogers. *Clin. Pharmacol. & Therap.* **7**, 740 (1966).
- ⁴ A. H. Beckett. *Progr. Drug Res.* **1**, 455 (1959).
- ⁵ J. W. Bellville and J. Green. *Clin. Pharmacol. & Therap.* **6**, 152 (1965).
- ⁶ H. Blumberg, H. B. Dayton and P. S. Wolf. *Fed. Proc.* **27**, 654 (1968).
- ⁷ O. J. Braenden, N. B. Eddy and H. Halbach. *Bull. Wld. Hlth. Org.* **13**, 937 (1955).

MECHANISMS OF ANALGESIA

- ⁸ A. Bossi, H. Besendorf, L. A. Pirk and A. Rheiner, Jr. *In Analgetics*, Ed. G. de Stevens, Academic Press, New York, 1965.
- ⁹ H. O. J. Collier, L. G. Dinneen, C. A. Johnson and C. Schneider. *Brit. J. Pharmacol. Chemother.* **32**, 295 (1968).
- ¹⁰ S. Courvoisier, R. Ducrot, J. Fournel and L. Julou. *C.R. Soc. Biol.* **151**, 1378 (1957).
- ¹¹ S. Courvoisier and O. Lean. *C.R. Acad. Sci.* **248**, 3227 (1959).
- ¹² F. E. D'Amour and D. L. Smith. *J. Pharmacol. Exp. Therap.* **72**, 74 (1941).
- ¹³ H. W. Elliott, N. Kokka and E. L. Way. *Proc. Soc. Exp. Biol. Med.* **113**, 1049 (1963).
- ¹⁴ W. O. Evans and B. A. Bergner. *J. New Drugs* **4**, 82 (1964).
- ¹⁵ F. N. Fastier. *Pharmacol. Rev.* **4**, 51 (1964).
- ¹⁶ R. A. Ferrari and J. D. Connolly. *Pharmacologist* **8**, 224 (1966).
- ¹⁷ H. F. Fraser. *Med. Clinics North America* **41**, 393 (1957).
- ¹⁸ H. F. Fraser and L. S. Harris. *Ann. Rev. Pharmacol.* **7**, 277 (1967).
- ¹⁹ H. F. Fraser and D. E. Rosenberg. *Clin. Pharmacol. & Therap.* **4**, 596 (1963).
- ²⁰ H. F. Fraser and D. E. Rosenberg. *J. Pharmacol. Exp. Therap.* **143**, 149 (1964).
- ²¹ R. F. Furchgott. *Pharmacol. Rev.* **4**, 21 (1964).
- ²² J. Garcia Leme and M. Rocha e Silva. *J. Pharm. Pharmacol.* **13**, 734 (1961).
- ²³ J. M. Glassman, F. Rauzzino, R. Goldstein and J. Mirsky. *Fed. Proc.* **27**, 653 (1968); N. Watzman and J. P. Buckley, *ibid.*; N. C. Campbell and I. Geller, *ibid.*
- ²⁴ J. D. Hardy, H. G. Wolff and H. Goodell. *Pain sensations and reactions*. Williams & Wilkins, Baltimore, 1952.
- ²⁵ R. A. Hardy, Jr. and M. G. Howell. *In Analgetics*, Ed. G. de Stevens, Academic Press, New York, 1965.
- ²⁶ L. S. Harris. *Arch. exp. Path. u. Pharmak.* **248**, 426 (1964).
- ²⁷ L. S. Harris and A. K. Pierson. *J. Pharmacol. Exp. Therap.* **143**, 141 (1964).
- ²⁸ L. S. Harris, A. K. Pierson, J. R. Dembinski and L. Dewey. *Arch. Int. Pharmacodyn.* **165**, 112 (1967).
- ²⁹ T. Jóhannesson and L. A. Woods. *Acta Pharmacol. Toxicol.* **21**, 381 (1964).
- ³⁰ T. Kakunaga, H. Kaneto and K. Hano. *J. Pharmacol. Exp. Therap.* **153**, 134 (1966).
- ³¹ T. G. Kantor, A. Sunshine, E. Laska, M. Meisner and M. Hopper. *Clin. Pharmacol. & Therap.* **7**, 447 (1966).
- ³² A. S. Keats and J. Telford. *J. Pharmacol. Exp. Therap.* **117**, 190 (1956).
- ³³ A. S. Keats and J. Telford. *J. Pharmacol. Exp. Therap.* **143**, 157 (1964).
- ³⁴ A. S. Keats and J. Telford. *Advan. Chem. Ser. No. 45*, 170, 1964.
- ³⁵ L. Lasagna and H. K. Beecher. *J. Pharmacol. Exp. Therap.* **112**, 356 (1954).
- ³⁶ L. Lasagna and T. J. DeKornfeld. *J. Am. Med. Assoc.* **178**, 887 (1961).
- ³⁷ R. K. S. Lim, F. Guzman, D. W. Rodgers, K. Goto, C. Braun, C. D. Dickerson, and R. J. Engle. *Arch. Int. Pharmacodyn.* **152**, 25 (1964).
- ³⁸ D. R. Maxwell, H. T. Palmer and R. W. Ryall. *Arch. Int. Pharmacodyn.* **132**, 60 (1961).
- ³⁹ M. Medaković and B. Banić. *J. Pharm. Pharmacol.* **16**, 198 (1964).
- ⁴⁰ J. W. Miller and H. W. Elliott. *J. Pharmacol. Exp. Therap.* **113**, 283 (1955).
- ⁴¹ S. J. Mulé, L. A. Woods and L. B. Mellett. *J. Pharmacol. Exp. Therap.* **136**, 242 (1962).
- ⁴² S. J. Mulé. *J. Pharmacol. Exp. Therap.* **148**, 393 (1965).
- ⁴³ J. G. Nutt. *Fed. Proc.* **27**, 753 (1968).
- ⁴⁴ P. D. Orahovats, C. A. Winter and E. G. Lehman. *J. Pharmacol. Exp. Therap.* **112**, 246 (1954).
- ⁴⁵ P. D. Orahovats, E. G. Lehman and E. W. Chapin. *J. Pharmacol. Exp. Therap.* **119**, 26 (1957).
- ⁴⁶ J. W. Pearson and T. J. DeKornfeld. *Anesthesiology* **24**, 38 (1963).
- ⁴⁷ L. O. Randall and J. J. Selitto. *Arch. Int. Pharmacodyn.* **111**, 409 (1957).
- ⁴⁸ A. D. Rudzik and J. H. Mennear. *J. Pharm. Pharmacol.* **17**, 326 (1965).
- ⁴⁹ O. Schaumann. *Brit. Med. J.* **2**, 1091 (1956).
- ⁵⁰ W. Schaumann. *Arch. exp. Path. u. Pharmak.* **235**, 1 (1958).
- ⁵¹ J. A. Schneider. *Proc. Soc. Exp. Biol. Med.* **87**, 614 (1954).
- ⁵² E. B. Sigg, G. Caprio and J. A. Schneider. *Proc. Soc. Exp. Biol. Med.* **97**, 97 (1958).
- ⁵³ R. I. Taber, D. D. Greenhouse and S. Irwin. *Nature* **204**, 189 (1964).
- ⁵⁴ H. Takagi, T. Takashima and K. Kimura. *Arch. Intern. Pharmacodyn.* **149**, 484 (1964).
- ⁵⁵ A. E. Takemori. *J. Pharmacol. Exp. Therap.* **135**, 252 (1962).
- ⁵⁶ J. Telford, C. N. Papadopoulos and A. S. Keats. *J. Pharmacol. Exp. Therap.* **133**, 106 (1961).

CHARLES A. WINTER

- ⁵⁷ J. Tripod and F. Gross. *Helv. Physiol. Pharmacol. Acta.* **15**, 105 (1957).
- ⁵⁸ R. A. Verri, F. G. Graeff and A. P. Corrado. *J. Pharm. Pharmacol.* **19**, 264 (1967).
- ⁵⁹ S. L. Wallenstein and R. W. Houde. *Fed. Proc.* **27**, 653 (1968).
- ⁶⁰ E. L. Way and T. K. Adler. *The biological distribution of morphine and its surrogates*, World Health Organization, Geneva, 1962.
- ⁶¹ B. Weiss and V. G. Latics. *J. Pharmacol. Exp. Therap.* **143**, 169 (1964).
- ⁶² C. V. Winder, M. Welford, J. Wax and D. H. Kaump. *J. Pharmacol. Exp. Therap.* **154**, 161 (1966).
- ⁶³ C. A. Winter. In *Analgetics*, Ed., G. deStevens, Academic Press, New York, 1965.
- ⁶⁴ C. A. Winter and L. Flataker. *J. Pharmacol. Exp. Therap.* **150**, 165 (1965).
- ⁶⁵ G. Woolfe and A. D. Macdonald. *J. Pharmacol. Exp. Therap.* **80**, 300 (1944).