THE MUSCLE PARALYZING ACTIONS OF ANTIBIOTICS

by
Y.N. SINGH, MSc, PhD, MIBiol
Lecturer in Chemistry
School of Natural Resources
University of the South Pacific, Suva.

INTRODUCTION

Although most of the side effects of antibiotics, e.g. nephrotoxicity, ototoxicity, neuropathology, hypersensitivity and allergic reactions had already been recognized, note was taken of their muscle paralysing actions only after Pridgen (1956)[1] reported the first clinical cases of prolonged respiratory depression following the intravenous administration of neomycin in conjunction with either anaesthesia. Subsequently a large number of antibiotics were implicated in cases of prolonged apnoea, usually when the patients had also received muscle relaxants such as tubocurarine[2,3], gallamine[4], or general anaesthetics[5].

The concurrent administration of relaxants and anaesthetics has been the most common precipitant of antibiotic-induced paralysis. However, other factors have also been involved:

a. route of administration, e.g. i.p. or i.v. instillation resulting in rapid absorption of the drug leading to a toxic concentration[6],

b. accidental overdosage[7],

c. therapeutic doses administered to patients with impaired renal function have led to accumulation of the antibiotic in toxic concentrations[8,9], and

d. use of antibiotics in patients with neuromuscular disorders e.g. Eaton-Lambert syndrome (myasthenic syndrome) and myasthenia gravis[10,11]

Experimental studies in animal preparations have not only confirmed these clinical reports but also have demonstrated that other antibiotics have muscle paralyzing actions. However, not all antibiotics have muscle paralyzing properties, even when used in conjunction with muscle relaxants or anaesthetics or in concentrations much higher than clinical doses. For example, bacitracin[12], the penicillins[13,14], cephalosporins and cephamycins[15,16] have been reported to lack neuromuscular blocking activity.

The antibiotics known to produce muscle paralysis may be classified chemically into 4 main groups:

(1) aminoglycosides (2) polymyxins (3) tetracyclines, and (4) lincosamides

In this review the mechanisms of action of these 4 groups of antibiotics will be considered.

THE AMINOGLYCOSIDES

Neuromuscular Effects

The following members of this group have been shown to posess neuromuscular blocking actions: amikacin[17,18], dihydrostreptomycin[19], gentamicin and kanamycin[20,21], neomycin[1,22], streptomycin[23] and tobramycin[24].

The aminoglycosides are all organic bases containing amino sugars linked to the hydroxyl groups of either streptidine or of its chemical congener deoxystreptamine[25]. It has been established that the streptidine and deoxystreptamine moieties are responsible for the neuromuscular paralysing actions of these antibiotics[26].

Mechanism of Action

The aminoglycosides do not depress directly elicited muscle twitches[23,27] and only affect nerve conduction at concentrations many times higher than those producing muscle paralysis[28,29], indicating that these compounds produce muscle paralysis primarily by interfering with neuromuscular transmission. The aminoglycosides produce a progressive flaccid paralysis of skeletal muscles with no initial facilitatory phase[20,29]. Thus, the actions of the aminoglycosides superficially resemble those of non-depolarizing neuromuscular blocking agents such as tubocurarine gallamine and pancuronium that are known to act primarily by blocking post-junctional acetylcholine receptors. However, neuromuscular block produced by tubocurarine - like drugs is well reversed by anticholinesterase drugs[30,31]. In contrast amino-

glycoside-induced neuromuscular block is well-reversed by calcium salts whereas calcium has very little effect on tubocurarine-induced block[18,31]. The observations cited above suggest that aminoglycosides act by a magnesium-like action.

Intracellular recording studies have confirmed the results of twitch-tension experiments. The main action of magnesium on neuromuscular transmission is to reduce the release of acetylcholine in response to nerve stimulation[32]. Magnesium is thought to act at the nerve terminal membrane by competing with calcium ions which are essential for the synchronous release of acetylcholine[33]. In such studies magnesium reduces the endplate potential (e.p.p.), i.e. the potential change in response to the evoked release of acetylcholine but only slightly and reduces the miniature endplate potentials (m.e.p.p.s.), i.e. the potential changes in response to the spontaneous release of individual packets, or quanta, of acetylcholine. Statistical analysis of these observations indicates that magnesium reduces the number of quanta of acetylcholine released by nerve stimulation, i.e. evoked acetylcholine release, and only slightly reduces postjunctional acetylcholine sensitivity[32]. Similar experiments with aminoglycoside antibiotics have shown that neomycin, gentamicin[34], streptomycin[26,35], amikacin[29] and the aminoglycoside - like agent spectinomycin[36] produce prejunctional effects similar to those of magnesium.

Inhibitory effects of aminoglycoside antibiotics on acetylcholine release have also been demonstrated by collecting and assaying the acetylcholine released either spontaneously or by nerve stimulation. Neomycin and gentamicin have been shown to have no effect on spontaneous release measured in this way[21] but neomycin[21,37], gentamicin[21] and streptomycin[37] all reduced the amount of acetylcholine released in response to nerve stimulation.

In addition to the evidence described above for a prejunctional action of aminoglycoside antibiotics and magnesium there is also evidence that these agents have some postjunctional effects. In the innervated rat hemidiaphragm equiactive neuromuscular blocking concentrations of neomycin, streptomycin and tubocurarine were found to depress responses to close intravenous injection of acetylcholine to differing degrees[37]. The predominantly postjunctionally active tubocurarine was more effective than either of the antibiotics.

The postjunctional actions of aminoglycosides including neomycin[34] streptomycin[35,38], amikacin[18] and spectinomycin[36] have also been investigated in intracellular recording studies and the aminoglycosides have been shown to reduce the amplitude of m.e.p.p.s. and/or responses to iontophoretically applied acetylcholine.

Magnesium, in addition to its prejunctional actions, possesses some postjunctional blocking action at high concentrations. However, despite the similarities between the pre- and postjunctional blocking actions of magnesium and the aminoglycosides it is unlikely that the postjunctional actions of either are mediated by competition with calcium ions as calcium itself has postjunctional blocking actions[39].

The relative contribution of the pre- and postjunctional components to the action of the aminoglycosides is difficult to assess, particularly in the clinical situation when the action of the antibiotics is likely to be superimposed on the action of other neuromuscular depressant drugs. Information from intracellular recording studies shows that postjunctional sensitivity tends to be reduced more than is evoked release at low antibiotic concentrations. However, the concentration-inhibition curve for inhibition of release is steeper than that for postjunctional sensitivity so that inhibition of release becomes more dominant as the concentration is increased[29,38].

THE POLYMYXINS

Neuromuscular Effects

The polymyxins consist of a series of chemically related cyclic compounds having a 7-membered polypeptide ring attached to a short polypeptide chain which terminates in a branched fatty acid, 6-methyloctanoic acid. The polypeptide chain and ring are made of 2,4-diaminobutyric acid and various amino acids so that the molecular weights of these antibiotics is approximately 1000. Polymyxins bind strongly to the phospholipid component of bacterial membranes destroying both membrane integrity and function and leading to death of the bacterium[40]. Besides the similarities in chemical structure, these compounds have been shown to possess pharmacological properties which are almost identical. They have very similar antimicrobial spectra, exhibit cross resistance and nephrotoxicity.

Members of the polymyxin group include polymyxins A,B,C,D,E (colistin), M and colistimethate (colistin methane sulphonate). Of these only polymyxin A, polymyxin B, colistin and colistimethate have been shown to produce neuromuscular block[12,29]. Unlike the aminoglycosides where the streptidine and deoxystreptamine moieties possess neuromuscular blocking properties, the intact polypeptide molecules of the polymyxins are necessary for activity as the individual components (amino acids, 2,4-diaminobutyric acid and 6-methyloctanoic acid) do not

produce neuromuscular blockage[12].

Polymyxins have been implicated in more than 30 reported cases of clinical paralysis[30], most of which occurred when the drugs were used with muscle relaxants or were associated with cases of renal dysfunction. In experimental studies the neuromuscular blocking properties of polymyxins are readily demonstrated and polymyxin B is the most potent neuromuscular blocking agent of all the antibiotics in current clinical use[12,31]. The order of neuromuscular blocking potencies of the polymyxins, which is also the order of antimicrobial potency[41], is polymyxin B > colistin > polymyxin A > colistimethate[12,31].

The neuromuscular blockade produced by polymyxins is augmented by tubocurarine and succinylcholine[23] and by pancuronium[42]. Calcium can reverse polymyxin-induced paralysis partially[30] or not at all[12,31]. The reported effects of anticholinesterases on polymyxin B-induced neuromuscular block also vary. In general the block is enhanced or prolonged[31,43,44].

Mechanism of action

Unlike the aminoglycosides, the polymyxins have depressant effects on muscle contractility as well as on neuromuscular transmission. Thus, in the isolated rat phrenic nerve-hemidiaphragm a concentration of polymyxin B sufficient to reduce responses to nerve stimulation by 95% also reduced responses to direct muscle stimulation by 50%[45]. The difference between the effects on the responses to the two types of stimulation is mainly due to inhibition of neuromuscular transmission. In both rat and mouse hemidiaphragm preparations the depression of neuromuscular transmission was readily reversed by washing but the action on muscle contractility was incompletely reversed[31,45]. Successive administrations of polymyxin B to the same preparation resulted in a gradual and irreversible reduction of muscle contractility In the clinical situation it is not known whether the neuromuscular depressant action or the action to depress contractility is responsible for the muscle paralysing actions of the polymyxins.

As far as the neuromuscular depressant actions of the polymyxins are concerned there is evidence for both pre- and postjunctional actions.

Neuromuscular blocking concentrations of polymyxin B have been shown to reduce evoked acetylcholine release in both mouse and frog preparations[29,35], although the depression of release was much less than that measured in the presence of equiactive neuromuscular blocking concentrations of streptomycin, amikacin, spectinomycin or magnesium.

In contrast to the results from intracellular recording studies, results from experiments involving the collection and assay of acetylcholine from isolated rat hemidiaphragm preparations have failed to show effects of colistin or polymyxin B on acetylcholine release[45].

Colistin and polymyxin B have been shown to depress responses of the rat hemidiaphragm to close intravenous injection of acetylcholine[45], and polymyxin B was also shown to depress contractural responses of the denervated rat hemidiaphragm[46]. McQuillen and Engbaek (1975)[47] found that colistimethate reduced m.e.p.p. amplitude and Singh et al. (1979)[35] found that muscle paralysing concentrations of polymyxin B completely abolished m.e.p.p. activity. Since Singh et al (1979)[35] had found that polymyxin B also reduced evoked acetylcholine release, they concluded that polymyxin B blocked neuromuscular transmission by a mixture of pre- and postjunctional actions.

Local anaesthetic action

Studies on desheathed frog sciatic nerve have shown the polymyxin B and the local anaesthetic lignocaine in equimolar concentrations had approximately equal effects on the extracellularly recorded gross nerve action potential[45]. At high pH (9.2) neither polymyxin B nor lignocaine had appreciable local anaesthetic activity indicating that, as for lignocaine[48], the charged form of polymyxin B is responsible for the local anaesthetic activity. The local anaesthetic activity of polymyxin B has also been demonstrated on intracellularly recorded muscle action potentials in the frog sartorius muscle in which the rates of rise and fall of the action potential were both decreased[29]. Hence, it is likely that the effects of polymyxin B on transmitter release and postjunctional acetylcholine sensitivity are at least as important factors as the local anaesthetic activity. Nevertheless, the local anaesthetic activity of polymyxin B could contribute to the observed depression of muscle contractility.

THE TETRACYCLINES

Neuromuscular Effects

Members of this group of antibiotics, which are all derivatives of polycyclic naphthacene carboxamide, are both chemically and pharmacologically distinct from the aminoglycosides, the polymyxins and the lincosamides[49]. At present, only 4 are commonly used in chemotherapy: chlortetracyline, oxytetracyline, rolitetracycline and tetracycline.

The ability of tetracyclines to produce neuromuscular paralysis in experimental animals is well established[31,45], but the number of reported clinical cases of

tetracycline-induced muscle paralysis is few. Rolitetracycline and oxytetracycline have produced neuromuscular paralysis, but only transiently, when given intravenously to patients suffering from myasthenia gravis[11].

The neuromuscular blocking action of tetracyclines in experimental animals was more pronounced during concomitant administration with tubocurarine[13], gallamine[50] or magnesium ions[51], the muscle paralysing actions of the tetracyclines were not consistently reversible by cholinesterase inhibitors or by calcium[11].

Mechanism of action

Although the tetracyclines have not been widely investigated, there is evidence to indicate that they might interfere with neuromuscular transmission. In the most comprehensive study of the muscle paralysing actions of the tetracylines before that of Singh (1979)[29], Wright and Collier (1976)[45] found that rolitetracycline reduced postjunctional receptor sensitivity but did not affect muscle contractility, nerve conduction or the release of acetylcholine. Although the actions of rolitetracycline resembled those of tubocurarine, the experimental evidence did not allow Wright and Collier to conclude that the two drugs acted by identical mechanisms.

In the mouse hemidiaphragm preparation a complete reversal by calcium of the block of indirectly elicited twitches produced by oxytetracycline was observed but very large concentrations of calcium were required to produce only a partial reversal of tetracycline induced neuromuscular block[31]. Intracellular recording studies showed that both tetracycline and oxytetracycline reduced evoked acetycholine release and the amplitude of m.e.p.p.s.These results show that the tetracyclines possess a mixture of pre- and postjunctional blocking actions.

However, in the mouse hemidiaphragm preparation, tetracycline and oxytetracycline had an action on muscle membrane or contractility which seemed to be as important as any neuromuscular blocking properties they might have. Although the local anaesthetic activity demonstrated for the tetracyclines may not be a major determinant in their actions at the neuromuscular junction, it may still be contributory to their effects on contractility.

The known ability of tetracyclines to chelate divalent ions, especially calcium, has led Pittinger and Adamson (1972)[52] to suggest that this action may explain the action of tetracyclines at the neuromuscular junction. Chelation would lower the extracellular calcium concentration and this in turn would lead to a reduction in acetylcholine release, as observed by Singh (1979)[29]. However, as this hypothesis is at variance with the results of Wright and Collier (1976)[45] it is, at the present time, impossible to make overall conclusions concerning the mechanism of action of the group of antibiotics. Further work in the future using the calcium ion selective electrode might clarify the situation.

THE LINCOSAMIDES

Neuromuscular Effects

Lincomycin and its semi-synthetic derivative clindamycin (7-deoxy 7-chloro lincomycin) are the only two of the various chemical congeners of lincomycin which are at present used in clinical practice.

Lincomycin-induced neuromuscular blockade has been demonstrated in various in vivo and in vitro experimental animal preparations[7,31,53]. In clinical use both lincomycin[54] and clindamycin[54,55], have been reported to prolong neuromuscular paralysis in patients after anaesthesia or treatment with muscle relaxants. Fogdall and Miller (1974)[55] reported that clindamycin phosphate given intravenously prolonged a pancuronium-induced neuromuscular blockade to about 20 hours in a patient who had previously shown normal sensitivity to pancuronium.

Despite the close structural similarity between lincomycin and clindamycin there are distinct differences in their muscle paralysing actions. Clindamycin is more effective than lincomycin against a wider variety of microorganisms in vitro and is much better absorbed from the gastrointestinal tract[56].

Lincomycin

Early experimental studies on lincomycin showed that the compound produces a blockade superficially similar to a non-depolarizing type in that flaccid paralysis is produced in chickens and no initial stimulation is seen in rabbits[7,54]. However, the blockade is not well antagonized by edrophonium, neostigmine or calcium[7,31].

The possibility that the anticholinesterase- and calcium-irreversible block produced by lincomycin might be due to a reduction of muscle contractility has been examined. In the isolated rat hemidiaphragm muscle Wright and Collier (1976)[53] showed that lincomycin reduced directly elicited muscle contractions in the same concentration range as that which reduced responses to nerve stimulation. These workers concluded that lincomycin reduced contractility but they were also able to show that in

combination with tubocurarine, low concentrations of lincomycin blocked neuromuscular transmission. In the isolated mouse hemidiaphragm, lincomycin appears to have very little inhibitory action on muscle contractility and its action is confined to the neuromuscular junction[31].

The mechanism of action of lincomycin at the neuromuscular junction has been further studied by intracellular recording and acetylcholine collection and assay techniques. Rubbo et al (1977)[54] found that subneuromuscular blocking concentrations of lincomycin increased the frequency of the spontaneous m.e.p.p.s. At neuromuscular blocking concentrations a depression of m.e.p.p. amplitude and of responses to iontophoretically applied acetylcholine was seen indicating a depression of postjunctional receptor sensitivity. Singh et al. (1979)[35] also found that m.e.p.p. amplitude was depressed by lincomycin but in addition, showed that evoked acetylcholine release, was depressed. These latter results on acetylcholine release are substantiated by results from collection and assay of acetylcholine[54]. Thus it can be concluded from the results of Singh et al.(1979)[35] and Rubbo et al.(1977)[54] that lincomycin possesses a mixture of pre- and postjunctional blocking activities. Attempts to reverse the neuromuscular block by a mixture of neostigmine and calcium were unsuccessful and hence it must be concluded that the pre-and postjunctional mechanisms of action are different from those of magnesium and tubocurarine respectively.

Clindamycin

In isolated nerve-muscle preparations clindamycin produces a marked increase in twitch tension before muscle paralysis ensues[31,53]. Similar initial increases in twitch tension have also been reported for lincomycin[53], streptomycin[57], tetracycline, rolitetracycline and oxytetracycline[31,45] and erythromycin[45].

In the case of clindamycin, following the initial increase in twitch tension there is a decrease in tension of both directly and indirectly stimulated preparations, indicating that the main cause of muscle paralysis is failure of contractility[31,53]. In general the effects of clindamycin are not reversed by anticholinesterases or calcium, suggesting that the junctional effects of clindamycin are less important than its action on muscle contractility.

Intracellular recording studies of the action of clindamycin on neuromuscular transmission show that the drug markedly increases spontaneous m.e.p.p.

frequency but at neuromuscular blocking concentrations also reduces their amplitude[35,54]. responses to ion-tophoretically applied acetylcholine are also reduced and this indicates that clindamycin possesses postjunctional receptor blocking activity[54]. Singh et al.(1979)[35] found that e.p.p. activity was difficult to measure and concluded that clindamycin induced failure of nerve terminal activity. Wright and Collier (1976)[53] have demonstrated that clindamycin possesses appreciable local anaesthetic activity and this action may be responsble for the failure of both nerve and muscle activity.

ACKNOWLEDGEMENTS:

The author's papers referred to in this review relate to the work done towards a PhD degree while he was an M.R.C. Research Assistant in the Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, Scotland. Help and cooperation of Dr. Alan Harvey and Dr. Ian Marshall are gratefully acknowledged.

REFERENCES:

1. Pridgen JE (1956) Surgery, 40 : 571-574.
2. Pittinger CB, Long JP and Miller JR (1958). Anesth. Analg., 37 : 276-282.
3. Bush GH (1962) Br. Med. J., 2 : 1062-1063.
4. Bodley PO and Brett JE (1962) Anaesthesia, 17 : 438-443.
5. Engel HL and Denson JS (1957) Surgery 42 : 862-864.
6. McQuillen ML Cantor HE and O'Rourke JR (1968) Arch. Neurol., 18 : 402-415.
7. Tang AH and Schroeder LA (1968) Toxicol. Appl. Pharmac., 12 : 44-49.
8. Bell RW and Jenicek JA (1966(Med. Ann. D. C., 35 : 603-604.
9. Pohlmann G (1966) J.A.M.A., 196 : 181-183.
10. Hokkanen E (1964) Br. Med. J., 1 : 1111-1112.
11. Gibbles E (1967) Deutsch. Med. Wochenschr., 92 : 1153-1154.
12. Adamson RH Marshall FN and Long JP (1960) Proc. Soc. Exp. Biol. Med., 105 : 494-497.
13. Bezzi G and Gessa L (1961) Antibiot. Chemother., 11 : 710-714.
14. Baisset A Lareng L and Puig G (1962) Anesth. Analg. (Paris), 19 : 813-825.
15. Ball AP, Gray JA and Murdoch JM (1975) Drugs, 10 : 81-111.
16. Marshall IG, Singh YN and Harvey AL (1979) J. Pharm. Pharmac., 31: 81p.
17. Hashimoto Y Shima T, Matsukawa S and Satou M (1978) Tohoku J. exp. Med., 125 : 71-75.
18. Singh YN, Marshall IG and Harvey AL (1978). Br. J. Anaesth., 50 : 109-117.
19. Jindal MN and Deshpande VR (1960) Bt. J. Pharmac., 15 : 506-509.
20. Vital Brazil O and Prado-Franceschi J (1969) Arch. int. Pharmacodyn. Ther., 179 : 65-77.
21. Vital Brazil O and Prado-Franceschi J (1969) Arch. int. Pharmacodyn. Ther., 179 : 78.85.
22. Pittinger CB and Long JP (1958) Antibiot. Chemother., 8 : 198-203.
23. Timmerman JC Long JP and Pittinger CB (1959). Toxicol. Appl. Pharmac., 1 : 299-304.
24. Waterman PM and Smith RB (1977) Anesth. Analg., 56 : 587-588.
25. Rinehart KL (1964) The Neomycins and Related Antibiotics, pp 40-114, John Wiley : New York.
26. Prado WA, Corrado AP and Marseillan RF (1978) Arch. int. Pharmacodyn. Ther. 231 : 297-307.
27. Vital Brazil O and Corrado AP (1957) J. Pharmac. exp. Ther., 120 : 452-459.
28. Diecke FPJ, Westecker ME and Vogt R (1971) Arch. int. Pharmacodyn. Ther., 193 : 5-13.
29. Singh YN (1979) Pharmacological Studies on the Muscle Paralyzing Actions of Antibiotics. PhD Thesis, University of Strathclyde.
30. Pittinger CB, Eryasa Y and Adamson R (1970) Anesth. Analg., 49 : 487-501.
31. Singh YN, Harvey AL and Marshall IG (1978) Anesthesiology, 48 : 418-424.
32. Del Castillo J and Engbaek L (1954) J. Physiol. (Lonf.), 124 : 370-384.
33. Dodge Jr DA and Rahamimoff R (1967) J. Physiol. (Lond.) 193 : 419-432.
34. Elmquist D and Josefsson JO (1962) Acta. Physiol- Scand., 54 : 105-110.
35. Singh YN, Marshall IG and Harvey AL (1979) Br. J. Anaesth., 51 : 1027-1033.
36. Singh YN, Marshall IG and Harvey AL (1979) Clin. exp. Pharmac. Physiol., 6 : 159-165.
37. Wright JM and Collier B (1977) J. Pharmac. exp. Ther., 200 : 576-587.
38. Dretchen KL, Gergis SD, Sokoll MC and Long JP (1972) Eur. J. Pharmac., 18 : 201-203.
39. Takeuchi N (1963) J. Physiol. (Lond.),167 : 141-155.
40. Newton BA (1956) Bacteriol. Rev., 20 : 14-27.
41. Nord NM and Hoeprich PD (1964) New Eng. J. Med., 270 : 1030-1035.
42. Burkett L, Bikhazi GB, Thomas KC et al (1979) Anesth. Analg., 58 : 107-115.
43. Fogdall RP and Miller RD (1974). Anesthesiology, 40 : 84-87.
44. Sabawala PB and Dillon JB (1959) Anesthesiology, 20 : 659-668.
45. Wright JM and Collier B (1976) Can. J. Physiol. Pharmac., 54 : 926-936.
46. Lullmann H and Reuter H (1960) Antibiotika Chemotherapia, 1 : 375-379.
47. McQuillen MP and Engbaek L (1975) Arch. Neurol., 32 : 235-238.
48. Ritchie JM Ritchie B and Greengard P (1965) J. Pharmac. exp. Ther., 150 : 160-164.
49. Laskin AI (1967) Antibiotics, Mechanisms of Action, ed. D. Gottlieb and P.D. Shaw, pp 331-339, Springer-Verlag: New York.
50. Bezzi G and Gessa GL (1960) Boll. Soc. Ital. Biol. Sper., 36 : 374-375.
51. Bowen JM and McMillan WC (1975) Am. J. Vet. Res., 36, 1025-1038.
52. Pittinger CB and Adamson R (1972) Ann. Rev. Pharmac., 12 : 169-184.
53. Wright JM and Collier B (1976) Can. J. Physiol. Pharmac., 54 : 937-944.
54. Rubbo JT Gergis SD and Sokoll MD (1977) Anesth. Analg., 56 : 329-332.
55. Fogdall RP and Miller RD (1974). Anesthesiology, 41 : 407-408.
56. Bartlett JG, Sutter VL and Finegold SM (1972) New Eng. J. Med., 287 : 1008-1010.
57. Dretchen KL, Sokoll MD, Gergis SD and Long JP (1973) Eur. J. Pharmac. 22 : 10-16.