Summary

The inhibition of total protein, DNA and isoprenoid synthesis by the potent T-2 toxin, was investigated in the murine macrophage cell line, J774. Protein and DNA synthesis were more than 50% inhibited within 1 hr by extremely low doses of the toxin (<1 ng/ml). Isoprenoid synthesis, unlike protein and DNA synthesis, showed delayed inhibition (i.e. after 3–4 hr). This effect of T-2 toxin on protein, DNA and isoprenoid biosynthesis presumably reflects the differential stability of the enzymes involved in these biosynthetic pathways.

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REFERENCES

1. Y. Ueno, in *Advances in Nutritional Research* (Ed. H. H. Draper), Vol. 3, p. 301. Plenum Press, New York (1980).
2. A. Ciegler, *J. Food Protec.* **41**, 399 (1978).
3. B. Yagen and A. Z. Joffe, *Appl. Microbiol.* **32**, 423 (1976).
4. I. Lutsky, N. Mor, B. Yagen and A. Z. Joffe, *Toxic. appl. Pharmac.* **43**, 111 (1978).
5. C. E. Mayer, *Milit. Surg.* **113**, 173 (1953).
6. A. Z. Joffe, in *Mycotoxic Fungi, Mycotoxin, Mycotoxicoses, An Encyclopedic Handbook* (Eds. T. D. Wyllie and L. G. Morehouse), Vol. 3, p. 21. Marcel Dekker, New York (1978).
7. C. S. McLaughlin, M. H. Vaughan, I. M. Cambell, C. M. Wei, M. E. Stafford and B. S. Hansen, in *Mycotoxins in Human and Animal Health* (Eds. J. V. Rodricks, G. W. Hesseltine and M. A. Mehlman), p. 263. Pathotox Publishers, Park Forest South, Illinois (1978).
8. Y. Ueno, M. Nakajima, K. Sakai, K. Ishii, N. Sato and N. Shimada, *J. Biochem. (Tokyo)* **74**, 285 (1973).
9. E. Morel-Chany, P. Burtin, G. Trincal and C. Frayssinet, *Masson (Paris)* **149**, (1980).
10. B. Yagen, A. Z. Joffe, P. Horn, N. Mor and I. I. Lutsky, in *Mycotoxins in Human and Animal Health* (Eds. J. V. Rodricks, G. W. Hesseltine and M. A. Mehlman), p. 329. Pathotox Publishers, Park Forest South, Illinois (1978).
11. P. Ralph and W. Nakoinz, *Nature* **257**, 393 (1975).
12. W. K. Cavenee, D. Johnston and G. Melnykovych, *Proc. natn. Acad. Sci. U.S.A.* **75**, 2103 (1978).
13. C. Lafarge-Frayssinet, G. Lespinats, P. Lafont, F. Loisillier, S. Mousset, Y. Rosenstein and C. Frayssinett, *Proc. Soc. exp. Biol. Med.* **160**, 302 (1979).
14. I. Kaneko, Y. Hazama-Shimada and A. Endo, *Eur. J. Biochem.* **87**, 313 (1978).
15. C. E. Agrelo and R. Schoental, *Toxic. Vet.* **5**, 155 (1980).
16. J. R. Faust, M. S. Brown and J. L. Goldstein, *J. biol. Chem.* **255**, 6546 (1980).
17. D. Barnes and G. Sato, *Cell* **22**, 649 (1980).
18. V. Quesney-Huneeus, M. H. Wiley and M. D. Siperstein, *Proc. natn. Acad. Sci. U.S.A.* **76**, 5056 (1979).

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Adrenergic ligand binding in human serum

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Propranolol is extensively bound in human serum and plasma [1–7]. *Alpha*-1 acid glycoprotein has been shown to be the major determinant for binding variability at therapeutic concentrations [2–8]. The binding of propranolol to this protein is saturable with high affinity [3, 9] and with stereospecificity [10, 11]. Other serum proteins have binding capacity for propranolol as well, but the binding to albumin and to lipoproteins has been reported to be nonsaturable [3, 4, 7]. A number of other adrenergic agents are bound to human serum proteins: alprenolol [5, 12], pindolol [5, 13, 14], timolol [5], oxprenolol [5, 15], prazosin [16], chlorpromazine [2, 7, 9], isoproterenol [1], epinephrine and norepinephrine [17–20].

The purpose of the present study has been to characterize the affinity and stereospecificity of adrenergic ligands in human serum by their ability to displace radiolabelled (−)-propranolol from its binding sites.

Materials and methods

Chemicals. [^{3}H]-(−)-Propranolol hydrochloride (sp. act. 19.6 Ci/mmole) was purchased from New England Nuclear, Dreieich, F.R.G. (−)-/(+)- and (±)-propranolol hydrochloride (Imperial Chemical Industries Ltd., London, UK), (−)- and (+)-alprenolol bitartrate (Haessle, Molndalen, Sweden), prazosin hydrochloride (Pfizer, Sandwich, U.K.) and (±)-hydroxybenzyl-pindolol (Sandoz A. G., Basel, Switzerland) were obtained as gifts. (−)- and (+)-Isoproterenol bitartrate, (−)-epinephrine bitartrate, (−)-norepinephrine bitartrate and ascorbic acid were purchased by Sigma Chemical Company (St. Louis, MO). Chlorpromazine and (±)-isoproterenol sulphate were purchased from the Norwegian Medicinal Depot, Oslo, Norway. All other chemicals were of analytical grade.

Buffers. Modified Krebs Ringer phosphate buffer: NaCl 122 mM, KCl 4.9 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.3 mM, Na_2HPO_4 15.9 mM, pH 7.40–7.45.

Modified Krebs Ringer bicarbonate buffer: NaCl 121 mM, KCl 4.8 mM, KH_2PO_4 1.2 mM $NaHCO_3$ 25.3 mM, $CaCl_2$ 1.3 mM, $MgSO_4$ 1.2 mM, pH 7.35–7.40 was achieved by gassing [5% (v/v) carbon dioxide in air].

Serum. Serum was obtained from healthy subjects. Venous blood was sampled after breakfast and left for 1 hr at room temperature and centrifuged at 2000 *g* for 30 min at 22°. Serum was aspirated immediately and stored at −20° until analysis.

Equilibrium dialysis. Serum protein binding of [^{3}H]-(−)-

propranolol was determined by equilibrium dialysis at 22° for 16–18 hr. In the experiments with unlabelled alprenolol, chlorpromazine, hydroxybenzylpindolol, prazosin and propranolol, 0.5 ml serum was dialyzed against 0.5 ml modified Krebs Ringer bicarbonate buffer, using a dialysis membrane 20/32 (Union Carbide Corp., Chicago, IL) clamped between two Perspex® cells. Dialysis was performed in an atmosphere of 5% (v/v) carbon dioxide in air to achieve pH 7.40–7.45. In the experiments with epinephrine, isoproterenol and norepinephrine 0.5 ml serum was dialyzed against 4.5 ml modified Krebs Ringer phosphate buffer, pH 7.40–7.45, using a dialysis membrane 32/32 (Medicell International Ltd., London, U.K.) clamped between two Perspex® cells. Before these experiments serum was dialyzed for 2 days at 4° against two shifts of modified Krebs Ringer phosphate buffer, pH 7.40–7.45. To prevent oxidation of the adrenergic amines, ascorbic acid was added to serum and buffer to obtain a final concentration of 0.4 mM. In addition, the Perspex® cells were flushed with pure nitrogen prior to dialysis.

In separate experiments we were not able to detect any differences in binding of propranolol by the various modifications of experimental procedures, including replacement of bicarbonate with phosphate in serum, the use of different buffers, ascorbic acid and the nitrogen atmosphere.

Serum concentration of radioligand. [^{3}H]-(−)-propranolol was added to serum to obtain an equilibrium concentration of 0.5 nM. After dialysis duplicate samples (50 μl) of serum or buffer were mixed with 10 ml scintillation liquid (Dilusolve®, Packard Instruments, Groningen, The Netherlands). The counting efficiency was 26–28% in both serum and buffer samples, determined in a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3330. The concentration of radioligand (in serum and buffer) could then be calculated from the specific activity of the radioligand.

Calculation of binding parameters. Protein binding of [^{3}H]-(−)-propranolol was expressed as the ratio B/F, B and F representing concentrations of bound and unbound ligand. The binding ratio, B/F, for the radioligand was constant at concentrations of unbound (±)-propranolol greater than 0.5 mM. We defined nonsaturable binding by this ratio and calculated saturable binding according to Chamness and McGuire [21].

Protein determinations. The concentration of total protein in serum was determined by the method of Lowry *et al.* [22] using bovine serum albumin as standard. Dilution during dialysis was between 10 and 20%

Results

Binding of (−)-propranolol. Labelled and unlabelled (−)-propranolol were added to serum to obtain unbound concentrations from 0.5 nM to 5 mM. Binding ratio, B/F, decreased with increasing concentration of unlabelled propranolol, with the minimum value of 0.4–0.7 obtained between 0.1 and 1 mM (Fig. 1). Increasing the concentration above 1 mM did not affect the binding ratio further. The same binding ratio was obtained with 0.5 mM (±)-propranolol (Fig. 2), and this concentration of racemic drug was used to define nonsaturable radioligand binding in human serum in the other experiments.

Inhibition of [^{3}H]*-(−)-propranolol binding by adrenergic antagonists.* Various concentrations of adrenergic antagonists were added to serum which contained an unbound radioligand concentration of 0.5 nM. As shown in Fig. 1, only small differences in the effect of the inhibitory ligands were observed. A considerable interindividual variability was observed in binding ratio (Fig. 2), but for all subjects the IC_{50}-value of saturable binding showed the same order of potency (Table 1): (±)-Hydroxybenzyl-pindolol > (−)-propranolol > (−)-alprenolol > prazosin = chlorpromazine. The presence of unlabelled ligands did not influence the nonsaturable binding (results not shown).

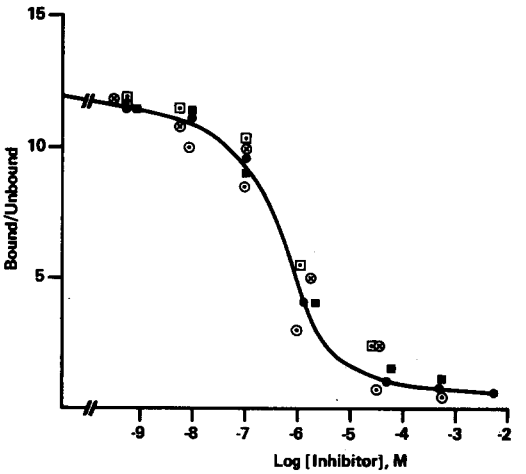

Fig. 1. Inhibition of total [^{3}H]-(−)-propranolol binding by (±)-hydroxybenzylpindolol (⊙), (−)-propranolol (●), (−)-alprenolol (■), prazosin (⊡) and chlorpromazine (⊗). The experimental procedures were performed as described in Methods. The results are presented as mean value from three separate experiments with different blood donors.

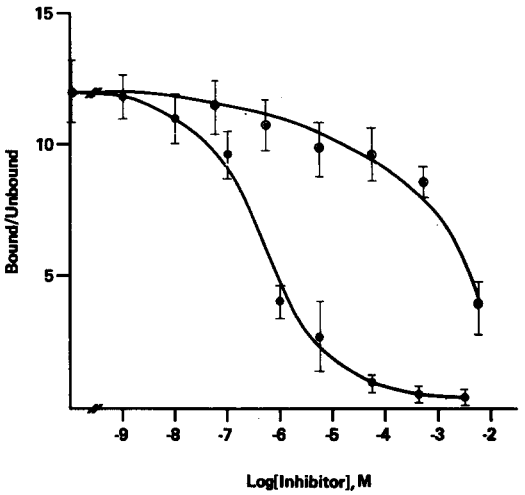

Fig. 2. Inhibition of total [^{3}H]-(−)-propranolol binding by (±)-propranolol ● and (±)-isoproterenol ⊙. The experimental procedures were performed as described in methods. The results are presented as mean value ± S.D. from three separate experiments with different blood donors.

Stereospecificity of propranolol and alprenolol binding. In order to characterize stereospecificity of the saturable binding site in serum, we tested the ability of (−)-, (±)- and (+)-propranolol and (−)- and (+)-alprenolol to inhibit radioligand binding. Figure 3 shows that the levoforms of both propranolol and alprenolol were more potent inhibitors of the radioligand binding than the dextroforms. At 50% inhibition the levoforms were 3–4 times more potent than the dextroforms. The dissociation constant for racemic propranolol was between that of the levo- and dextroform (Table 1).

Inhibition of [^{3}H]*-(−)-propranolol binding by (±)-isoproterenol.* Different concentrations of (±)-isoproterenol were added to serum which contained an unbound radiolabelled (−)-propranolol concentration of 0.5 nM. Figure

Table 1. Dissociation constants (K_I) for adrenergic antagonists determined by inhibition of saturable [^{3}H]-(−)-propranolol binding. The experiments and calculations were performed as described in the Methods, and the concentration causing half maximal saturable radioligand binding (IC_{50}) represents under the present experimental conditions K_I. The results are presented as mean value ± S.D. from three separate experiments with different blood donors

Antagonist	Dissociation constant (K_I) (μM) Mean ± S.D.	Range
(±)-Hydroxybenzylpindolol	0.1 ± 0.02	0.08–0.1
(−)-Propranolol	0.4 ± 0.1	0.3–0.5
(±)-Propranolol	0.8 ± 0.3	0.6–1.0
(+)-Propranolol	1.4 ± 1.1	0.7–3.2
(−)-Alprenolol	0.7 ± 0.1	0.5–0.8
(+)-Alprenolol	2.1 ± 1.3	1.0–3.2
Prazosin	1.0 ± 0.8	0.6–2.0
Chlorpromazine	1.3 ± 0.9	0.6–2.0

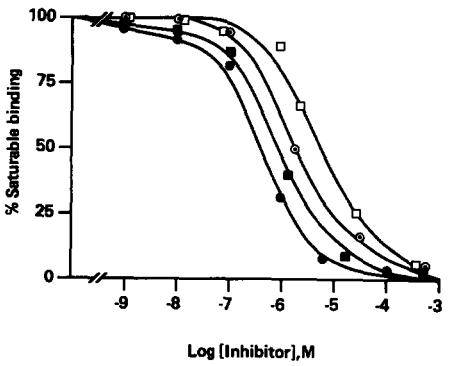

Fig. 3. Stereospecific inhibition of saturable [^{3}H]-(−)-propranolol binding by propranolol and alprenolol. The experimental procedures and calculations were as described in methods. The results are presented as mean value from three different experiments with different blood donors. (−)-Propranolol (●), (+)-propranolol ⊙, (−)-alprenolol (■) and (+)-alprenolol (□).

2 shows that inhibition of radioligand binding increased with increasing agonist concentration, but at a considerably higher concentration than (±)-propranolol.

Order of agonist inhibitory potency. To determine the ability of adrenergic agonists to interact with the saturable propranolol binding site in serum, we determined radioligand binding in presence of various concentrations of agonists. For all subjects the IC_{50}-value of saturable binding showed the same order of potency: (−)-Isoproterenol > (−)-norepinephrine > (−)-epinephrine and stereospecificity existed: (−)-Isoproterenol > (±)-isoproterenol > (+)-isoproterenol (Table 2). The *alpha*-adrenergic agonist, phenylephrine, did not inhibit the saturable binding of radioligand.

Discussion

The binding of propranolol to serum proteins is extensively characterized. *Alpha*-1 acid glycoprotein is the major binding protein at therapeutic concentrations [2–8] with one saturable binding site characterized by high affinity [3, 9]. It has been shown that propranolol is associated with other serum proteins, but in contrast to the binding site on *alpha*-1 acid glycoprotein, the binding to serum albumin and to serum lipoproteins is nonsaturable [3, 4, 7]. Accordingly, total serum binding can be decomposed into saturable and nonsaturable binding of radiolabelled propranolol.

The saturable serum binding of propranolol was characterized by high affinity. The dissociation constant for racemic propranolol is in accordance with previous reported values [3, 7]. We found that propranolol was stereospecifically bound with a ratio between the dextro- and levoform of approximately 3.5 at 50% inhibition of radioligand binding. Stereospecificity for propranolol was also reported recently and *alpha*-1 acid glycoprotein was shown to account for the stereospecificity in plasma [10, 11].

Alprenolol is bound in human serum to a similar or somewhat lower extent than propranolol [5, 12], but inhibited radiolabelled propranolol binding stereospecifically with a ratio between the dextro- and levoform of approximately 3.0. Alprenolol and propranolol are, in addition to disopyramid and verapamil the only basic drugs bound to *alpha*-1 acid glycoprotein [23, 24] that presently have been shown to exhibit stereospecificity [25, 26]. On the other hand, the acid drug warfarin is stereospecifically bound to human serum albumin [27].

Pindolol is bound less extensively in serum than propranolol [5, 13, 14], but the racemic form of hydroxybenzylpindolol had higher affinity for the saturable binding site than the levoform of propranolol. This can probably be explained by an increase in binding affinity of hydroxybenzylpindolol by introduction of the hydroxybenzyl group which also increases the affinity of the ligand to the *beta*-adrenergic receptors [28].

Table 2. Dissociation constants (K_I) for adrenergic agonists determined by inhibition of saturable [^{3}H]-(−)-propranolol binding. The experiments and calculations were performed as described in the Methods, and the concentration causing half maximal saturable radioligand binding (IC_{50}) represents under the present experimental conditions K_I. The results are presented as mean value ± S.D. from three separate experiments with different blood donors

Agonist	Dissociation constant (K_I) (mM) Mean ± S.D.	Range
(−)-Isoproterenol	0.9 ± 0.2	0.7–1.1
(±)-Isoproterenol	2.3 ± 0.8	1.9–3.2
(+)-Isoproterenol	4.0 ± 0.5	3.5–4.5
(−)-Norepinephrine	4.6 ± 0.7	4.0–5.3
(−)-Epinephrine	9.8 ± 1.7	8.1–12

The *alpha*-adrenergic blocker prazosin was a strong inhibitor of saturable radioligand binding suggesting that *alpha*-1 acid glycoprotein is an important binding protein for this ligand. The dissociation constant in the present study is in close correspondence with the value in an earlier report [16]. Chlorpromazine has, in addition to other effects, strong *alpha*-adrenergic blocking properties [29] and is highly bound in serum or plasma, mainly by *alpha*-1 acid glycoprotein [2, 7, 9]. The dissociation constant, determined by inhibition of saturable propranolol binding in the present study, is similar to that reported earlier [7, 9]. In all experiments we found the same order of potency for the ability to inhibit saturable propranolol binding: (±)-Hydroxybenzylpindolol > (−)-propranolol > (−)-alprenolol > prazosin = chlorpromazine.

The serum protein binding of isoproterenol is markedly lower than for propranolol [1]. In accordance to this, we found that isoproterenol had low affinity for the saturable propranolol binding site in serum, but inhibited radioligand binding stereospecifically: (−)-Isoproterenol > (±)-

isoproterenol > (+)-isoproterenol. For the related adrenergic amine, norepinephrine, both absence [18, 19] and presence [20] of stereospecificity has been reported.

Isoproterenol was the most potent inhibitor among the agonists with (−)-norepinephrine more potent than (−)-epinephrine, in accordance with an earlier study [20]. However, norepinephrine has also been reported to be less [17] or equally [18] bound in human serum as epinephrine. The differences in experimental procedures may explain the different results. Our observations indicate that the adrenergic agonists have affinity for the binding site on *alpha*-1 acid glycoprotein. Phenylephrine, classified as an *alpha*-adrenergic agonist, had no ability to inhibit radiolabelled propranolol binding.

The saturable binding site for propranolol in serum, probably mainly located on *alpha*-1 acid glycoprotein, has similarities to the *beta*-adrenergic receptor binding site: The antagonists are bound with considerably higher affinity than the agonists, both agonists and antagonists exhibit stereospecific binding, and the *beta*-adrenergic ligands were more potent than the *alpha*-adrenergic substances. However, great differences in binding affinity and specificity between the saturable binding site in serum and the *beta*-adrenergic receptor rule out the possibility of a solubilized and intact circulating receptor.

In the present study we have decomposed serum binding of radiolabelled propranolol into saturable and nonsaturable binding. The nonsaturable binding was not affected by the addition of other adrenergic ligands, in contrast to saturable binding that was inhibited both by antagonists and agonists. The order of inhibitory potency was: (±)-Hydroxybenzylpindolol > (−)-propranolol > (−)-alprenolol > prazosin = chlorpromazine for the antagonists and (−)-Isoproterenol > (−)-norepinephrine > (−)-epinephrine > (−)-phenylephrine for the agonists. The antagonists had markedly higher affinity for the binding site compared to the agonists, But stereospecificity was found for both antagonists and agonists with the levoform markedly more potent than the dextroform. Studies are in progress to characterize the common adrenergic binding site in human serum.

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REFERENCES

1. J. G. Kelly and D. G. McDevitt, *Br. J. clin. Pharmac.* **6**, 123 (1978).
2. K. M. Piafsky, O. Borgå, I. Odar-Cederlöf, C. Johansson and F. Sjöqvist, *N. Engl. J. Med.* **299**, 1435 (1978).
3. G. Sager, O. G. Nilsen and S. Jacobsen, *Biochem. Pharmac.* **28**, 905 (1979).
4. S. Glasson, R. Zini, P. d'Athis, J.-P. Tillement and J. R. Boissier, *Molec. Pharmac.* **17**, 187 (1980).
5. F. M. Belpaire, M. G. Bogaert and M. Rosseneu, *Eur. J. clin. Pharmac.* **22**, 253 (1982).
6. J. W. Paxton and R. L. Calder, *J. Pharmac. Meth.* **10**, 1 (1983).
7. R. K. Verbeeck, J.-A. Cardinal, A. G. Hill and K. K. Midha, *Biochem. Pharmac.* **32**, 2565 (1983).
8. B. J. Scott, A. R. Bradwell, R. E. Schneider and H. Bishop, *Lancet* **i**, 930 (1979).
9. W. E. Müller and A. E. Stillbauer, *Naunyn-Schmiedeberg's Arch. Pharmac.* **322**, 170 (1983).
10. U. K. Walle, T. Walle, S. A. Bai and L. S. Olanoff, *Clin. Pharmac. Ther.* **34**, 718 (1983).
11. F. Albani, R. Riva, M. Contin and A. Baruzzi, *Br. J. clin. Pharmac.* **18**, 244 (1984).
12. K. M. Piafsky and O. Borgå, *Clin. Pharmac. Ther.* **22**, 545 (1978).
13. E. A. Taylor and P. Turner, *Br. J. clin. Pharmac.* **12**, 543 (1981).
14. M. Lemaire and J.-P. Tillement, *Biochem. Pharmac.* **31**, 359 (1982).
15. A. Sioufi, D. Hillion, P. Lumbroso, R. Wainer, M. Olivier-Martin, J. P. Schoeller, D, Colussi, F. Leroux and P. Mangoni, *Br. J. clin. Pharmac.* **18**, 453 (1984).
16. P. Rubin and T. Blasche, *Br. J. clin. Pharmac.* **9**, 177 (1980).
17. G. Franksson and E. Änggård, *Acta pharmac. tox.* **28**, 209 (1970).
18. A. Danon and J. D. Sapira, *J. Pharmac. exp. Ther.* **182**, 295 (1972).
19. J. C. Russell and D. M. Doty, *Physiol. Chem. Physics* **5**, 75 (1973).
20. G. Powis, *Biochem. Pharmac.* **24**, 707 (1975).
21. G. C. Chamness and W. L. McGuire, *Steroids* **26**, 538 (1975).
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. chem.* **193**, 265 (1951).
23. J. J. Lima, H. Boudoulas and M. Blanford, *J. Pharmac. exp. Ther.* **219**, 741 (1981).
24. F. X. McGowan, M. J. Reiter, E. L. C. Pritchett and D. G. Shand, *Clin. Pharmac. Ther.* **33**, 485 (1983).
25. J. J. Lima, G. L. Jungbluth, T. Devine and L. W. Robertson, *Life Sci.* **35**, 835 (1984).
26. M. Eichelbaum, G. Mikus and B. Vogelgesang, *Br. J. clin. Pharmac.* **17**, 453 (1984).
27. E. M. Sellers and J. Koch-Weser, *Pharmac. Res. Commun.* **7**, 331 (1975).
28. P. A. Insel and L. M. Stoolman, *Molec. Pharmac.* **14**, 549 (1978).
29. B. M. Cohen, M. Herschel and A. Aoba, *Psych. Res.* **1**, 199 (1979).

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Competitive binding of the oximes HI-6 and 2-PAM with regional brain muscarinic receptors

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Most organophosphate (OP) poisoning has been treatable with a combination of an anti-muscarinic compound, such as atropine, and an oxime, such as [({[(4-aminocarbonyl)-pyridino]methoxy)methyl]-2-[(hydroxyimino)methyl] pyridinium dichloride (HI-6) [1, 2]. The oximes probably penetrate the blood–brain barrier [3, 4], as both indirect [5]