Biochemical Pharmacology, Vol. 25, pp. 2073–2077, Pergamon Press, 1976. Printed in Great Britain.

INHIBITION OF MONOAMINE OXIDASE BY AMPHETAMINE AND RELATED COMPOUNDS

TIMOTHY J. MANTLE, KEITH F. TIPTON and NIGEL J. GARRETT
Department of Biochemistry, Tennis Court Road, Cambridge CB2 1QW, England

(*Received* 24 *January* 1976, *accepted* 7 *April* 1976)

Abstract—The α-methyl-substituted amines *dl*-α-methylbenzylamine, *dl*-α-methyltryptamine and the two stereoisomers of amphetamine were shown to be competitive inhibitors of the oxidation of benzylamine, tyramine and serotonin by rat liver monoamine oxidase. All these compounds were more potent inhibitors of serotonin oxidation than of benzylamine oxidation, with α-methyltryptamine showing the greatest selectivity and α-methylbenzylamine the least. The kinetics of the inhibition of tyramine oxidation were consistent with the presence of two enzyme species with different inhibitor sensitivities which were both active towards this substrate. The selectivity of these inhibitors was demonstrated with membrane-bound and solubilised preparations of the enzyme, but it could be abolished by treatment of the latter preparation with the chaotropic agent sodium perchlorate. The significance of monoamine oxidase inhibition in connexion with the pharmacological action of amphetamine is discussed.

The pharmacological action of amphetamine is believed to be due, at least in part, to its ability to increase the concentrations of transmitter amines in the synaptic cleft. This effect involves stimulation of catecholamine release from the nerve endings [1–5] and inhibition of reuptake into the nerve endings [6–10]. The relative importance of these two effects is uncertain and may vary with the nature of the transmitter amine, since differing concentrations of amphetamine have been shown to be necessary to cause the release of different transmitter amines [11, 12].

Although amphetamine is a reversible inhibitor of monoamine oxidase [13], it has been generally assumed that this does not play a significant role in the pharmacological effects of this compound since the inhibition is relatively weak [14] and the effects of amphetamine differ from those produced by monoamine oxidase inhibitors (see e.g. [15]). Glowinski *et al.* [16] showed that the inhibition of monoamine oxidase by amphetamine was dependent on the substrate used to assay the enzyme, tryptamine oxidation being less sensitive to inhibition than the activity towards moradrenaline. A similar dependence of the inhibitor constant upon the substrate used to assay the enzyme was noted by Fuller [17], and Green [18] reported that the inhibition of tyramine oxidation varied with amphetamine concentration in a way that suggested that more than one enzyme species was responsible for the oxidation of this substrate. None of these workers, however, presented the results of a detailed kinetic study in which a range of substrate and inhibitor concentrations was used and in this paper we report the results of such a study with the enzyme from rat liver, using the two stereoisomers of amphetamine and the α-methyl derivatives of benzylamine and tryptamine.

MATERIALS AND METHODS

[1-^{14}C]-labelled serotonin creatinine sulphate and tyramine hydrochloride were obtained from the Radiochemical Centre, Amersham, Bucks, U.K. and [1-^{14}C] benzylamine hydrochloride was obtained from ICN Pharmaceuticals, Irvine, Calif., U.S.A. *d*- and *l*-amphetamine sulphate, from Smith, Kline and French Laboratories Inc., were a gift from Dr. L. L. Iversen, and *dl*-α-methyltryptamine hydrochloride was a gift from Dr. R. F. Long of Roche Products Ltd, Welwyn Garden City, Herts, U.K., *dl*-α-methylbenzylamine was obtained from Ralph N. Emmanuel Ltd, Wembley, Middx. U.K. and was converted to its hydrochloride and recrystallised before use. All other chemicals were obtained from British Drug Houses Ltd, Poole, Dorset, U.K.

Enzyme preparations. Rat liver mitochondrial outer membranes and solubilised partly purified preparations of monoamine oxidase were prepared by the methods previously reported [19, 20] and treatment of the solubilised preparation with the chaotropic agent sodium perchlorate followed the procedure previously described [19]. Beef liver aldehyde dehydrogenase was prepared by a modification [19] of the procedure of Deitrich *et al.* [21].

Assay methods. The activity of monoamine oxidase was followed at 30° by using the radiochemical method of Otsuka and Kobayashi [22], the coupled spectrophotometric assay with aldehyde dehydrogenase [19] or by monitoring the oxygen uptake using an oxygen electrode (Rank Bros, Bottisham, Cambridge, U.K.). Assays were carried out either in 0.1 M sodium phosphate buffer or 50 mM Tris–HCl buffer at pH 7.2 with no other additions to the assay except for enzyme, substrate and inhibitor. No difference could be detected between the activities in these two buffers.

RESULTS

Assay of the activity of the monoamine oxidase in rat liver mitochondrial outer membranes and the solubilised preparation using the oxygen electrode confirmed that none of the α-methyl derivatives could function as substrates for the enzyme. In addition the

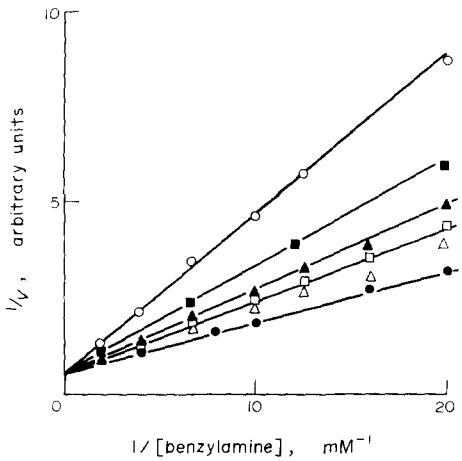

Fig. 1. Double-reciprocal plots of the inhibition of benzylamine oxidation by *l*-amphetamine using rat liver mitochondrial outer membranes as the enzyme source. Experimental details were as described in the text and the following *l*-amphetamine concentrations were used: 0 (●), 0.1 (△), 0.25 (□), 0.5 (▲), 0.75 (■) and 1.0 (○) mM.

possibility of activity towards α-methylbenzylamine was investigated spectrophotometrically at 240 nm since the acetophenone that would be produced by the oxidation of this compound absorbs strongly at this wavelength [24]. There was no evidence for the production of any material absorbing at 240 nm when either the solubilised or the membrane-bound enzyme was incubated with α-methylbenzylamine at 10 mM, 1 mM or 0.1 mM for periods of up to 60 min.

The enzyme preparations were shown to be inhibited competitively by all the α-methyl-amines with tyramine, benzylamine and serotonin as the assay substrated. In all cases inhibitor studies were carried out using both the radiochemical assay [22] and the coupled assay [19] and the results obtained from these two methods were found to be in agreement within experimental error. Figs. 1–3 show double-reciprocal plots for the inhibition of the activity in rat

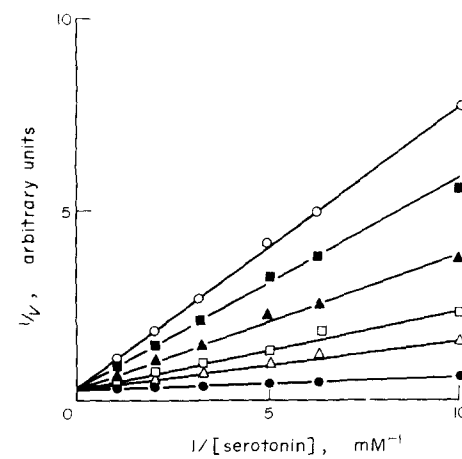

Fig. 2. Double-reciprocal plots of the inhibition of serotonin oxidation by *l*-amphetamine using rat liver mitochondrial outer membranes as the enzyme source. Experimental details were described in the text and the following *l*-amphetamine concentrations were used: 0 (●), 0.1 (△), 0.25 (□), 0.5 (▲), 0.75 (■) and 1.0 (○) mM.

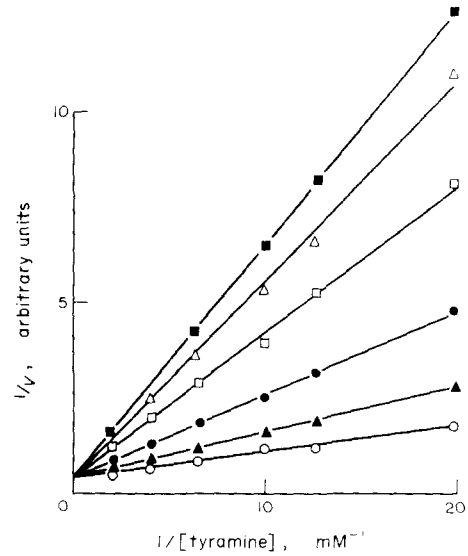

Fig. 3. Double reciprocal plots of the inhibition of tyramine oxidation by *l*-amphetamine using rat liver mitochondrial outer membranes as the enzyme source. Experimental details were as described in the text and the following *l*-amphetamine concentrations were used: 0 (○), 0.1 (▲), 0.2 (●), 0.5 (□), 0.75 (△) and 1.0 (■) mM.

liver mitochondrial outer membranes by *l*-amphetamine using the three different substrates. Secondary plots of the slopes of these double-reciprocal plots against the concentration of the inhibitor were linear with all the inhibitors when benzylamine or serotonin was the substrate (see e.g. Fig. 4) but with the solubilised and membrane-bound enzyme preparations the inhibitor constant (K_i) was lower when serotonin was used as the substrate.

Secondary plots for the inhibition of tyramine oxidation by each of the α-methyl derivatives were hyperbolic (see e.g. Fig. 5) when either the solubilised or the membrane-bound preparation of the enzyme was used. This behaviour could be due to the presence of more than one enzyme species with different inhibitor sensitivities which were both active towards

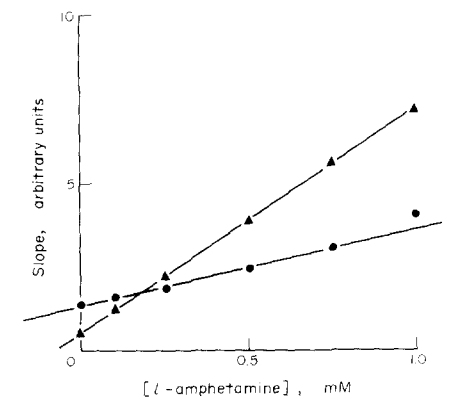

Fig. 4. Secondary plots for the inhibition of the monoamine oxidase activity towards benzylamine and serotonin by *l*-amphetamine. The slopes of the lines in Figs. 1 and 2 are plotted against the concentration of *l*-amphetamine. ● Benzylamine oxidation. ▲ Serotonin oxidation.

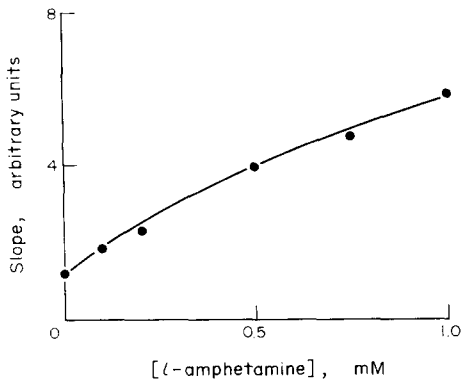

Fig. 5. Secondary plots for the inhibition of monoamine oxidase activity towards tyramine by *l*-amphetamine. The slopes of the lines in Fig. 3 are plotted against the concentration of *l*-amphetamine. The solid line results from a theoretical calculation assuming that 60% of the enzyme activity had a K_i value of 600 μM and the remainder had a K_i value of 70 μM, with the K_m values of both enzyme species towards tyramine being the same.

this substrate. Previous studies using selective irreversible inhibitors have indicated that rat liver monoamine oxidase contains two species of activity that will oxidase tyramine with about 40 per cent of this activity being also active towards serotonin but not benzylamine and 60 per cent being active towards benzylamine but not serotonin (see e.g. [25–27]). The curve in Fig. 5 is a theoretical plot of the results that would be expected if the oxidation of tyramine were due to the presence of two such species with 60 per cent of the activity having the K_i value shown towards benzylamine as the substrate and the remainder having the K_i value obtained with serotonin as the substrate (see Table 1).

After the solubilised enzyme had been treated with the chaotropic agent sodium perchlorate, the inhibition by the α-methyl amines showed no significant selectivity towards any of the substrates tested and secondary plots of the inhibition data obtained when tyramine was the substrate were linear in all cases, as shown for the inhibition by *l*-amphetamine in Fig. 6.

The K_i values obtained with these inhibitors and the three different enzyme preparations with benzylamine, serotonin and tyramine as the substrates are

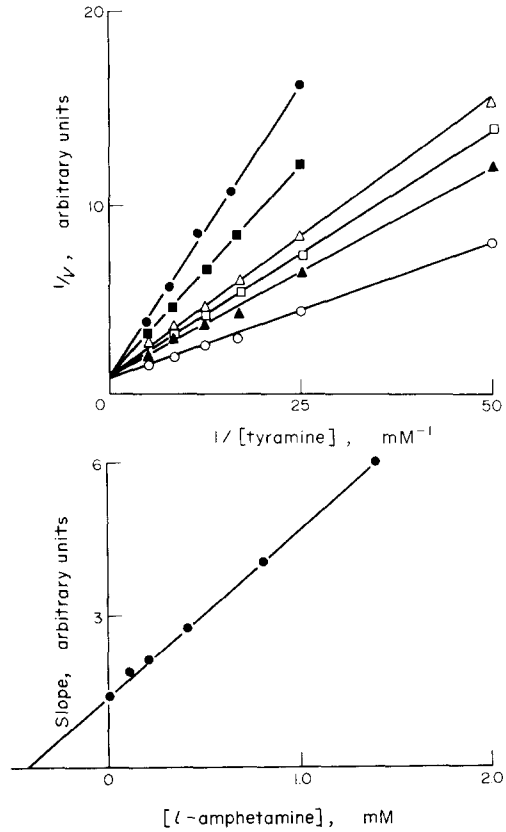

Fig. 6. Inhibition of tyramine oxidation by *l*-amphetamine using the perchlorate-treated enzyme preparation. Experimental details are given in the text. (A) Double-reciprocal plots at the following *l*-amphetamine concentrations: 0 (○), 0.1 (▲), 0.2 (□), 0.4 (△), 0.8 (■) and 1.4 (●) mM. (B) Secondary plot of the slopes of the lines in Fig. 6A against the concentration of *l*-amphetamine.

shown in Fig. 1. It can be seen that, of the inhibitors tested, α-methyltryptamine showed the greatest selective potency towards serotonin as a substrate and α-methylbenzylamine showed the least.

DISCUSSION

Since Long [28] first reported selective inhibition of monoamine oxidase in 1962, a variety of compounds have been shown to act as selective reversible

Table 1. Inhibition of rat liver monoamine oxidase by α-methyl-substituted amines

Enzyme preparation	Substrate	K_i (μM)			
		d-Amphetamine	*l*-Amphetamine	α-Methylbenzylamine	α-Methyltryptamine
Membrane-bound	Benzylamine	770	600	650	1250
	Serotonin	20	70	120	0.5
	Tyramine	N.L.	N.L.	N.L.	N.L.
Solubilised	Benzylamine	580	760	700	1850
	Serotonin	60	160	300	8
	Tyramine	N.L.	N.L.	N.L.	N.L.
Perchlorate-treated	Benzylamine	430	500	250	500
	Serotonin	480	480	340	540
	Tyramine	440	430	330	490

N.L. indicates that secondary plots of the inhibition data were non-linear. Details of the experimental methods used are given in the text.

or irreversible inhibitors of the enzyme (see e.g. [17, 26, 27, 29, 38]). The current evidence indicates that, in many species, the enzyme can be regarded as being composed of two major forms which were termed the A and B species by Johnston [29]. The specificities of these two forms in rat liver have been studied and benzylamine and phenethylamine have been shown to be substrates for the B species, serotonin has been shown to be a substrate for the A species and tyramine has been shown to be a substrate for both species (see e.g. [25–27, 29]). The situation with respect to tryptamine is unclear, since it has been classified as a substrate for the B species in some studies [26] but as a substrate for both species in others [25, 27]. There is evidence that the properties of monoamine oxidase from rat liver may represent an adequate model for the enzyme from rat and human brain, since the proportions of the two major enzyme species and their specificities appear to be similar in preparations from these sources (compare the results in [26, 31, 33 and 34]).

With the possible exception of α-methyltryptamine, the inhibitors used here can be regarded as α-methyl analogues of the B species substrates benzylamine and phenethylamine and thus their greater potency as inhibitors of the A species is surprising, although Housley and Tipton [26] have shown that the substrates for one of the enzyme species are able to bind to the other species with K_i values similar to their K_m values when acting as substrates. Substrates for the B enzyme species are, however, mixed inhibitors of the A species whereas the analogues used here were all competitive inhibitors. The competitive inhibition by the amphetamines is in agreement with the results of Fuller [17] and Green [18] although neither of these workers presented a complete analysis of the dependence of inhibition on the inhibitor concentration and the former author did not use a sufficient range of inhibitor concentrations to detect the non-linear dependence on inhibitor concentration. Mantle *et al.* [30] found non-linear secondary plots for inhibition of tyramine oxidation by α-methyltryptamine but they did not investigate the inhibition of the activity towards other substrates. The agreement between the experimental points shown in Fig. 5 and the theoretical curve drawn by assuming that the activity towards tyramine was due to 60 per cent species A activity and 40 per cent B activity with the K_i values shown by these species, when the activity was assayed with serotonin and benzylamine respectively, provides further evidence that tyramine is a substrate for both species and is in agreement with the conclusion reached by Mantle *et al.* [30] who used a somewhat different model.

There is evidence that the apparent multiplicity of monoamine oxidase in rat liver and human brain may be due to a single enzyme species existing in different membrane lipid environments [19, 31], and treatment of solubilised preparations of the enzyme with chaotropic agents, such as sodium perchlorate, has been shown to abolish the apparent multiplicity of the enzyme, including the selective responses to inhibitors, without loss of activity [19, 31]. In agreement with these results treatment of the solubilised preparation of the enzyme with sodium perchlorate abolished the selectivity of the inhibitors studied here and also the evidence that two enzyme species were involved in the activity towards tyramine. A comparison of the K_i values for these inhibitors with the membrane-bound and solubilised enzyme preparations shows that solubilisation itself produces changes in the sensitivity of the enzyme which is in agreement with previous results that have shown that solubilisation produces changes in the kinetic mechanism [32] and the inhibitor sensitivity [33] of the enzyme.

The selectivity of amphetamine inhibition towards the A species is of interest since this species has been shown to be responsible for the oxidation of noradrenaline as well as serotonin [26, 34]. Since inhibition of monoamine oxidase in nerve endings will result in inhibition of the reuptake process as a consequence of rising levels of free intraneuronal amines [35], it is tempting to ascribe inhibition of reuptake to this effect. However, little difference has been noted between the inhibitory effect on noradrenaline and dopamine reuptake in rat brain [12], although this model would predict a weaker effect on dopamine reuptake since it is a substrate for both enzyme species [26, 34]. In addition *d*-amphetamine has been reported to be a relatively weak inhibitor of serotonin uptake [36].

The difference between the selectivity of the two isomers of amphetamine are in accord with the greater potency shown by the *d*-isomer in its pharmacological effects. Sandler and Reynolds [37] have recently suggested that, in view of the structural similarity between amphetamine and phenethylamine, one might regard phenethylamine as being a naturally occurring amphetamine analogue. Since amphetamine overdosage may produce symptoms similar to schizophrenia, they argued that this disease might be associated with an abnormal phenethylamine response. The results of the present study indicate that, in the case of monoamine oxidase inhibition, amphetamine does not act as a phenethylamine analogue but rather as an analogue of substrates for the species A enzyme.

It appears from the results reported here that substitution of a methyl group at the α-position of substrates for monoamine oxidase produces reversible inhibitors which are selective for the A species. The situation with regard to irreversible inhibitors is, however, less easy to define since pheniprazine and tranylcypromine, which can in a sense both be regarded as amphetamine analogues, show little selectivity [17] and the more extensively substituted compound Deprenyl is a selective inhibitor of the B species [38].

Acknowledgements K. F. T. is grateful for a grant from the Medical Research Council in support of this work. The assistance of a grant from the Smith, Kline and French Foundation at the earlier stages of this work is also gratefully acknowledged. We are grateful to Dr. R. F. Long and Dr. L. L. Iversen for their kind gifts of some of the inhibitors used in these studies.

REFERENCES

1. M. J. Besson, A. Cheramy, P. Feltz and J. Glowinski, *Proc. natn. Acad. Sci. U.S.A.* **62**, 741 (1969).
2. R. J. Boakes, P. B. Bradley and J. M. Candy, *Br. J. Pharmac.* **45**, 391 (1972).

3. L. A. Carr and K. E. Moore, *Science* **64,** 322 (1969).
4. K. Y. Ng, T. N. Chase and I. J. Kopin, *Nature, Lond.* **228,** 468 (1970).
5. R. J. Ziance, A. J. Azzaro and C. O. Rutledge, *J. Pharmac. exp. Ther.* **182,** 284 (1972).
6. M. J. Beeson, A. Cheramy, P. Feltz and J. Glowinski, *Brain Res.* **32,** 407 (1971).
7. A. J. Azzaro, R. J. Ziance and C. O. Rutledge, *J. Pharmac. exp. Ther.* **189,** 110 (1974).
8. M. R. Ferris, F. L. M. Tang and R. A. Maxwell, *J. Pharmac. exp. Ther.* **181,** 407 (1972).
9. J. Glowinski and J. Axelrod, *J. Pharmac. exp. Ther.* **149,** 43 (1965).
10. P. F. Von Voigtlander and K. E. Moore, *J. Pharmac. exp. Ther.* **184,** 542 (1973).
11. A. J. Azzaro and C. O. Rutledge, *Biochem. Pharmac.* **22,** 2801 (1973).
12. M. Raiteri, A. Bertollini, F. A. Angelini and G. Levi, *Eur. J. Pharmac.* **34,** 189 (1975).
13. H. Blaschko, D. Richter and H. Schlossman, *Biochem. J.* **31,** 2817 (1937).
14. C. L. Zirkle and C. Kaiser, in *Psychopharmacological Agents* (Ed. M. Gordon) Vol. 1, p. 445. Academic Press, New York (1964).
15. L. Stein, *Fedn Proc.* **23,** 836 (1964).
16. J. Glowinski, J. Axelrod and L. L. Iversen, *J. Pharmac. exp. Ther.* **153,** 30 (1966).
17. R. W. Fuller, *Archs Int. Pharmacodyn.* **174,** 32 (1968).
18. A. L. Green, *Biochem. J.* **121,** 37P (1971).
19. M. D. Houslay and K. F. Tipton, *Biochem. J.* **135,** 173 (1973).
20. M. D. Houslay and K. F. Tipton, *Biochem. J.* **135,** 735 (1973).
21. R. A. Deitrich, L. Hellerman and J. Wein, *J. biol. Chem.* **237,** 560 (1962).
22. S. Otsuka and Y. Kobayashi, *Biochem. Pharmac.* **13,** 995 (1962).
23. K. F. Tipton and A. P. Dawson, *Biochem. J.* **108,** 95 (1968).
24. C. S. Vestling and M. C. Rebstock, *J. biol. Chem.* **161,** 285 (1945).
25. D. W. R. Hall, B. W. Logan and G. H. Parsons, *Biochem. Pharmac.* **18,** 1447 (1969).
26. M. D. Houslay and K. F. Tipton, *Biochem. J.* **139,** 645 (1974).
27. T. J. Mantle, K. Wilson and R. F. Long, *Biochem. Pharmac.* **24,** 2031 (1975).
28. R. F. Long, *Acta neurol. scand.* **38,** S1, 27 (1962).
29. J. P. Johnston, *Biochem. Pharmac.* **17,** 1285 (1968).
30. T. J. Mantle, K. Wilson and R. F. Long, *Biochem. Pharmac.* **24,** 2039 (1975).
31. K. F. Tipton, M. D. Houslay and N. J. Garrett, *Nature, Lond.* **246,** 213 (1973).
32. M. D. Houslay and K. F. Tipton, *Biochem. J.* **145,** 311 (1975).
33. K. F. Tipton, M. D. Houslay and T. J. Mantle, Ciba Foundation Symposium: *Monoamine Oxidase and its Inhibitors*, in press.
34. N. H. Neff and H-Y. T. Yang, *Life Sci.* **14,** 2061 (1974).
35. U. Trendelenburg, P. R. Draskoczy and K. H. Graefe, *Adv. Biochem. Psychopharmac.* **5,** 371 (1972).
36. D. T. Wong, J. S. Horng and R. W. Fuller, *Biochem. Pharmac.* **22,** 311, (1973).
37. M. Sandler and G. P. Reynolds, *Lancet* I, 70 (1976).
38. J. Knoll and K. Magyar, *Adv. Biochem. Psychopharmac.* **5,** 393 (1972).