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Effects of polysorbates and Cremophor EL on vascular responses in rat aorta

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Abstract. The effects of Tween 20, Tween 80, and Cremophor EL, surface active agents which are used for dispersion of water-insoluble substances, on vascular responsiveness were investigated using rat aortic rings. In high concentrations all these agents produced persistent contractions in aortic rings independent of the presence of endothelium. These contractions were not influenced by inhibitors of known endogenous contractile mediators. Incubation with these agents caused a concentration-dependent inhibition of the endothelium-dependent relaxant responses to acetylcholine in intact precontracted aortic rings. Endothelium-independent relaxations produced by sodium nitroprusside were not inhibited, but rather potentiated in the presence of Tween 80 (10^{-1} ml/l). On the other hand, Tween 80 inhibited the contractile effects of 5-hydroxytryptamine, phenylephrine, and bradykinin significantly. The data suggests that these substances affect both endothelial cells and vascular smooth muscle.
Key words. Tweens; Cremophor EL; rat aorta; vascular responsiveness.

Polysorbates, non-ionic surface active agents (SAAs), and Cremophor EL (Cre EL) are frequently used as solubilization aids for water-insoluble substances in a wide variety of medicinal products designed for either topical or systemic use. Although these substances were reported to be of somewhat low toxicity despite the rather high concentrations used, some doubt has arisen in the last decade over their suitability for use in medicinal products as excipients[1–3]. There have been reports of adverse reactions to either Cre EL[4–12] or Tween 80[13–23] in humans and animals. These substances are also used in experiments to test the role of water-insoluble substances in biological responses. Ideally, solvents used for these purposes should have no pharmacological effect. It has not yet been clarified whether these agents affect responses to vasoactive substances. In a preliminary study we noticed that Tween 80, in an acceptable concentration as a solubilization adjuvant, abolished the relaxation mediated by endothelium. Thus, the present study was undertaken to determine the effect of SAAs on endothelium-dependent as well as independent vascular responses.

Materials and methods

Locally bred albino rats (220–280 g) of either sex were anaesthetized with sodium pentobarbital (30 mg/kg) and sacrificed by exsanguination from the common carotid arteries. Thoracic aortae were quickly isolated, cleaned of connective tissue, and cut into transverse rings 3–4 mm width. The rings were fixed vertically between stainless steel wire hooks and suspended under 2 g of resting tension in a tissue bath containing 10 ml of Krebs-bicarbonate solution at 37 °C, continuously aerated with 95% O_2 + 5% CO_2. The upper wire was connected to a Grass FT-03 force displacement transducer for isometric force measurements. Tissues were equilibrated for 60 min, during which the medium was replaced every 15 min. In some experiments, endothelium was removed by gently rolling the rings on wires, and the effectiveness of this deendothelization procedure was determined by the abolition of relaxation induced by 10^{-6} M acetylcholine (ACh). Composition of Krebs-bicarbonate solution was as follows (millimolar): NaCl 118; KCl 4.7; $CaCl_2$ 1.5; $NaHCO_3$ 25; $MgSO_4$ 1.1; KH_2PO_4 1.2; and glucose 5.6.

To study the endothelium-dependent responses, tissues were precontracted with a predetermined concentration of phenylephrine (5×10^{-7}–10^{-6} M) which caused 75–80% of the maximal contraction. After reaching a plateau they were relaxed by cumulative concentrations of ACh. After washing for 15 min and recovery for 30 min, tissues were incubated for 30 min with various concentrations of Tween 80 (T-80), Tween 20 (T-20), or Cremophor EL (Cre EL). Aortic rings were then precontracted again and tested for ACh-induced relaxation in the presence of SAAs. Each ring was exposed to only a single concentration of one of these SAAs. Results were expressed as percentage of relaxation of Phe-induced tone. In order to determine the effects of SAAs on contractile responses, concentration-response curves of phenylephrine (Phe), 5-hydroxytryptamine (5-HT), or bradykinin (Bk) were obtained before and after incubation with T-80 (10^{-1} ml/l) in endothelium-denuded preparations. To test the effects of T-80 on endothelium-independent relaxation, responses to sodium nitroprusside (SNP) were examined in endothelium-denuded rings precontracted with Phe.

All values within each figure were calculated as percentage of maximal contraction or relaxation. Results are expressed as means ± SEM. EC50 values for agonists were defined as the concentration of agent producing 50% of the individual maximum effect and are expressed as geometric means. Statistical significance was examined by using Student's *t*-test for paired or unpaired observations.

Drugs: All drugs except UK 38485 (3-(1H-Imidazol-1-yl methyl)-2-methyl-1H-indole-1-propanoic acid, Pfizer) used in this study were obtained from Sigma Chemical (St Louis, MO).

Results

Effects of SAAs on basal tension. All three SAAs at the high concentration (1 ml/l) produced persistent contractions of rat aortic rings regardless of whether endothelium was present or absent. This contraction was not affected by repeated washing, or by antagonists or inhibitors such as phentolamine (alpha-adrenoceptor antagonist, 10^{-6} M), diphenhydramine (histamine-1 receptor antagonist, 10^{-5} M), indomethacin (cyclooxygenase inhibitor, 10^{-5} M), UK 38485 (thromboxane synthase inhibitor, 10^{-5} M), and phosphoramidone (kininase II and endothelin converting enzyme inhibitor, 10^{-7} M) (data not shown). At the high concentration (1 ml/l) of T-20 or T-80, aortic rings were further contracted with Phe, but no relaxation with ACh was observed in endothelium intact preparations. In the case of Cre EL (1 ml/l), ACh produced endothelium-dependent relaxation in an inhibited manner. Relatively lower concentrations of SAAs (10^{-1}–10^{-5} ml/l) produced no change in the base-line tone, so we limited our studies to the analysis of inhibition of endothelium-dependent relaxation to low concentrations of these compounds.

Effects of SAAs on ACh-induced relaxation. In intact aortic rings precontracted submaximally with Phe, addition of ACh (10^{-8}–10^{-4} M) produced concentration-dependent relaxation. In preliminary control experiments there was no detectable difference between two concentration-response curves of ACh obtained 30 min apart. Exposure of these rings to various concentrations of T-20, T-80 or Cre EL (10^{-1}–10^{-5} ml/l) attenuated the ACh-induced relaxations significantly and dose-dependently (fig. 1). There were also significant decreases in the maximum relaxation responses to ACh in the presence of SAAs (table 1). Additionally, they produced significant and concentration-dependent changes in concentration of ACh required to produce 50% of maximum relaxation (EC 50). The potency order of SAAs was as follows: T-80 > T-20 > Cre EL. T-80 was found to be the most effective at a concentration of 0.1 ml/l for shifting the −log EC 50 value of ACh from 7.20 ± 0.08 to 3.29 ± 0.04, whereas Cre EL shifted the −log EC 50 value of ACh only slightly.

Effects of T-80 on endothelium-independent relaxations induced by SNP. In Phe-precontracted aortic rings denuded of endothelium, SNP produced concentration-dependent relaxation. However, relaxation responses to lower concentrations of SNP (10^{-10}–3×10^{-8} M) in the presence of T-80 (0.1 ml/l) were found to be significantly greater than control relaxation (fig. 2). Although T-80 did not change the maximum relaxation to SNP, −log EC 50 values of SNP were found to be significantly

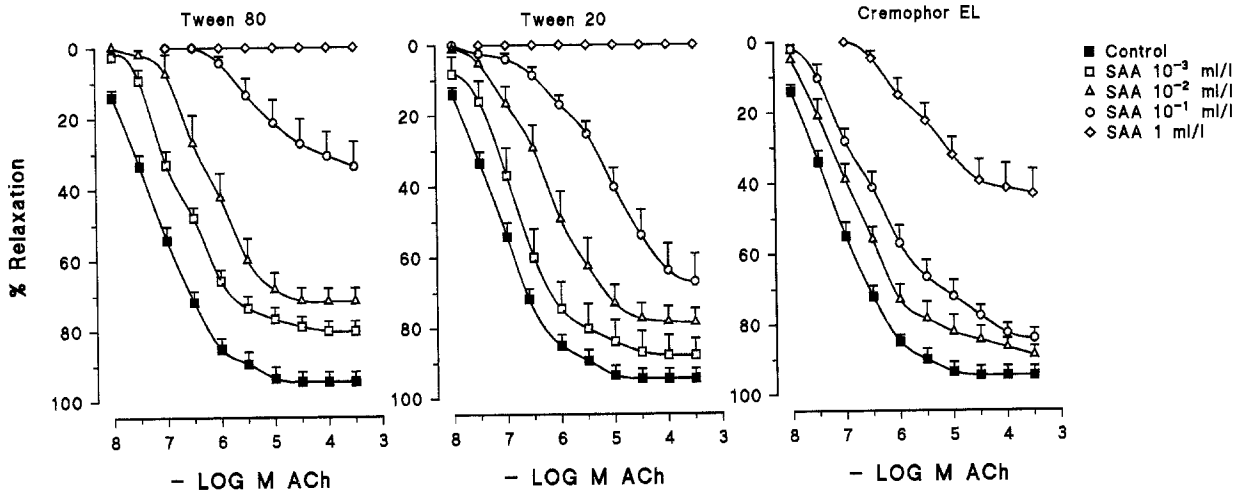

Figure 1. Concentration-response curves showing the effects of Tween 80, Tween 20, and Cremophor EL (10^{-3}–1 ml/l) on endothelium-dependent relaxation evoked by acetylcholine (ACh) in intact rat aortic rings precontracted with Phe. Each artery ring was exposed to only a single concentration of one of these surface active agents (SAAs). Relaxation is expressed as the percentage of decrease in Phe-induced tone. Each point is the mean of 8–10 observations; vertical bars indicate the SEM. For the sake of clarity symbols of significance are omitted. Filled symbols represent the control values. Open symbols indicate that the difference between relaxations before and after treatment with SAA is statistically significant (Student's *t*-test for paired observations; a probability of 0.05 or less was accepted as significant).

Table 1. EC 50 values and maximum responses of acetylcholine (ACh) in presence of surface active agents (SAAs).

Treatment (ml/l)		EC 50 ($-\log M \pm SEM$)		Maximum response (% ± SEM)	
		control	SAA	control	SAA
Tween 80	10^{-3}	7.30 ± 0.07	6.69 ± 0.07**	94.7 ± 3.7	80.2 ± 4.6*
	10^{-2}	7.12 ± 0.08	6.07 ± 0.07**	91.5 ± 4.6	71.7 ± 3.8*
	10^{-1}	7.20 ± 0.08	3.29 ± 0.04**	94.7 ± 5.1	37.8 ± 9.9*
	1	7.06 ± 1.06	-	91.8 ± 3.9	T.I.
Tween 20	10^{-3}	7.21 ± 0.08	6.49 ± 0.08**	91.6 ± 7.4	83.4 ± 6.6*
	10^{-2}	7.11 ± 0.07	6.10 ± 0.06**	92.3 ± 4.9	78.9 ± 4.4*
	10^{-1}	7.02 ± 0.06	4.36 ± 0.05**	93.3 ± 4.7	67.9 ± 8.0*
	1	7.49 ± 1.19	-	94.2 ± 5.2	T.I.
Cre EL	10^{-3}	7.59 ± 0.10	7.54 ± 0.09	94.0 ± 4.1	91.5 ± 4.1
	10^{-2}	7.51 ± 0.09	7.04 ± 0.05**	96.5 ± 3.1	87.7 ± 2.1*
	10^{-1}	7.59 ± 0.10	5.70 ± 0.06**	94.0 ± 3.8	84.0 ± 3.1*
	1	7.51 ± 0.09	4.22 ± 0.06**	96.6 ± 2.3	43.1 ± 3.8*

Each value represents 8–10 experiments, T.I. = Total inhibition.
*$p < 0.05$, **$p < 0.001$, significantly different from corresponding control value. Student's t-test for paired observations.

Table 2. EC 50 values and maximum responses of contractile agents in presence of Tween 80 (T-80: 10^{-1} ml/l).

	EC 50 ($-\log M \pm SEM$)		Maximum response (gram ± SEM)	
	control	T-80	control	T-80
Phe	7.21 ± 0.07	6.82 ± 0.06*	0.959 ± 0.109	0.867 ± 0.098*
5-HT	7.17 ± 0.08	6.74 ± 0.07*	0.907 ± 0.086	0.745 ± 0.074*
BK	4.59 ± 0.06	4.52 ± 0.07	0.535 ± 0.059	0.388 ± 0.068*

Each value represents 8–12 experiments. *$p < 0.005$, significantly, different from corresponding control value. Student's t-test for paired observations.

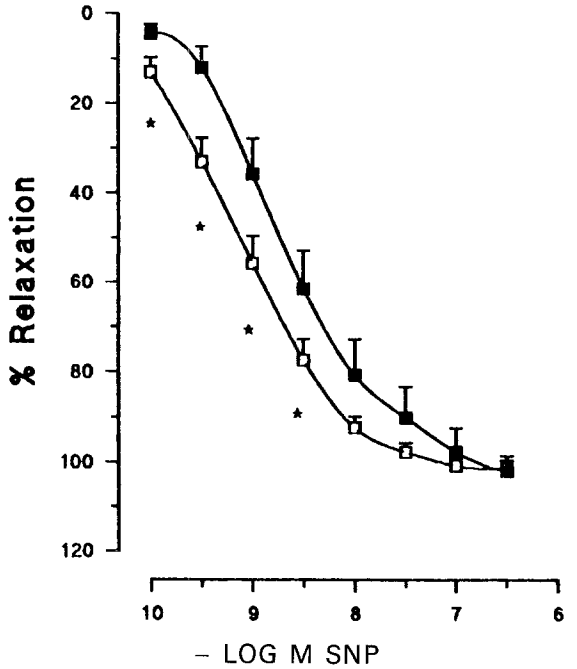

Figure 2. Concentration-response curves showing the effect of Tween 80 (10^{-1} ml/l) on endothelium-independent relaxation evoked by SNP (10^{-10}–10^{-7} M) in deendothelized rat aortic rings precontracted submaximally with Phe. Relaxations are expressed as the percentage of decrease in Phe-induced tone. Each point is the mean of 8–10 observations; vertical bars indicate the SEM. Statistically significant from corresponding control value *$p < 0.05$ (Student's t-test for paired observations).

different (8.67 ± 0.07 and 9.13 ± 0.08 before and after T-80 treatment, respectively).

Effects of T-80 on contractile responses in deendothelized aortic rings. In endothelium-denuded preparations, Phe (10^{-8}–10^{-5} M), 5-HT (10^{-9}–10^{-5} M) and Bk (10^{-5}–3×10^{-4} M) all caused concentration-dependent contractions. Incubation with T-80 at a concentration of 0.1 ml/l for 30 min shifted the concentration-response curves of these agonists to the right (fig. 3). T-80 reduced the maximum contractile responses to these agonists by approximately 20%. Additionally, EC 50 values of Phe and 5-HT were found to be increased in the presence of T-80 by the ratio of 3.0 and 2.5, respectively (table 2).

Discussion

Polysorbates and Cre EL are frequently used for dispersion of water insoluble drugs in some medicinal products. The inhibitory effect of Cre EL, a Cyclosporin A solvent, on endothelium-dependent relaxations has been shown in rabbit mesenteric artery and thoracic aorta[24]. It is reported that Cre EL acts as a weak thromboxane A_2-receptor agonist, or alternatively its vasoactive property is due to the small amount of ricinoleic acid that might be present in the Cre EL[25]. The interactions between polysorbates and endothelium-dependent re-

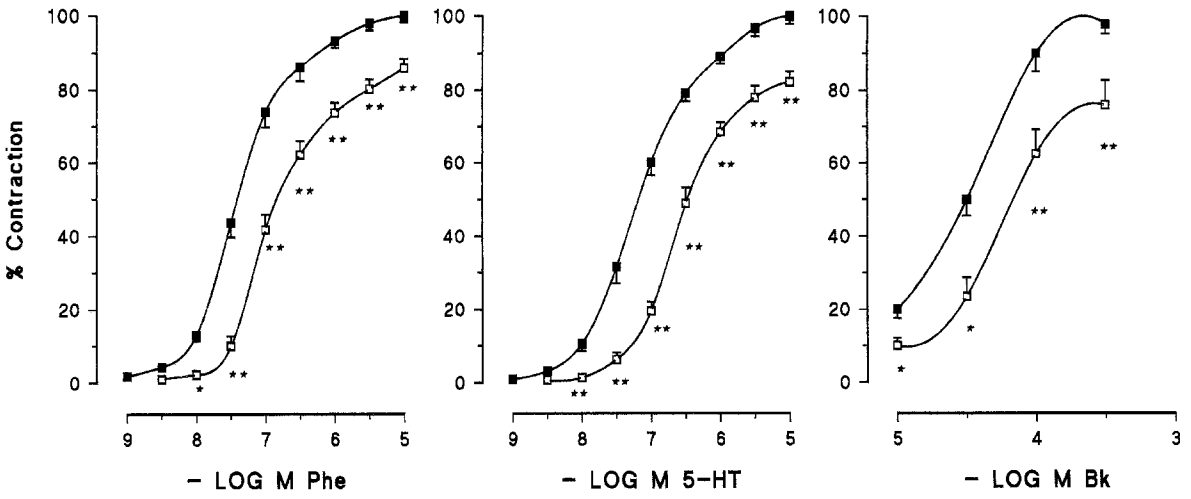

Figure 3. Concentration-response curves showing the effect of Tween 80 (10^{-1} ml/l) on contractions induced by Phe (10^{-9}–10^{-5} M), 5-HT (10^{-9}–10^{-5} M), and Bk (10^{-5}–3×10^{-4} M) in rat aortic rings denuded of endothelium. Results are expressed as the percentage of maximal contraction for each agonist. Filled symbols represent the control values, and open symbols represent the contractions evoked by agonists in the presence of Tween 80. Each point is the mean of 10–12 observations; vertical bars indicate the SEM. Statistically significant from corresponding control value *$p < 0.05$, **$p < 0.005$ (Student's t-test for paired observations).

sponses have not been extensively studied. In a study using rats injected with T-80 at a concentration of 10 ml/kg i.p. weekly for 4 weeks, no significant changes were reported either in the histopathological examination of endothelium or in the pulmonary responsiveness to ACh or Angiotensin II[26]. Although functional or histological damage of endothelial cells was not demonstrated with T-80 treatment[26], it appears from the present study that acutely administered polysorbates and Cre EL impair the functional status of endothelial cells, since the relaxation responses to ACh in precontracted aortic rings were inhibited. Our data show that incubation of deendothelized aortic rings with T-80 does not inhibit the relaxation response to SNP, the endothelium-independent vasorelaxant, but rather potentiates it at lower concentrations of SNP. This suggests that SAAs show an inhibitory effect on relaxation only when the relaxation is endothelium-dependent. Both polysorbates and Cre EL at the high concentration produced a considerable increase in the base-line tension of aortic rings regardless of the presence of endothelium. The fact that phentolamine, diphenhydramine, indomethacin, UK 38485, and phosphoramidone did not show any effect suggests that the probable release of endogenous mediators of smooth muscle contraction is not involved in this tension increase caused by SAAs in the high concentration.

In the present study, the inhibitory effects of polysorbates on ACh-mediated relaxation were similar to those induced by Cre EL. Thus, these agents may share a common inhibitory mechanism. Consistent with our findings, it has been reported that perfusion of rat mesenteric arterial bed with some other surface active detergent substances such as sodium deoxycholate or CHAPS (3-((3-cholamidopropyl)-dimethylammonio)-1-propanesulphonate) resulted in a reduction of endothelial function[27–29]. In the present study, T-80 was found to be the most effective in this manner when similar concentrations of SAAs were compared.

Although SAA treatment decreased the effectiveness of ACh as an endothelium-dependent vasorelaxant, it is also possible that it is the sensitivity of smooth muscle rather than the activity of the endothelium that has been affected. In fact, endothelium-independent concentration of aortic rings observed at higher concentrations of SAAs suggested a direct action of these agents on vascular smooth muscle. T-80 significantly inhibited the contractile responses of Phe, 5-HT and Bk as well. The inhibition of contractile responses with the lack of inhibition of SNP-induced relaxations points out a mechanism(s) other than a change in the sensitivity of the smooth muscle.

Therefore, we could not conclude anything about the precise mechanism of these reductions in the effects of either ACh or vasoconstrictors. Whether the effect of SAAs is due to a change in the morphological character of smooth muscle or endothelial cells, or in a step related to functional status, remains to be investigated.

In conclusion, the present results show that T-20, T-80 and Cre EL inhibit the potency of ACh as an endothelium-dependent vasorelaxant, and also the contractile effects of vasoactive agents. Such actions must be taken into account when using SAAs as solubilizing aids.

1 Reynolds, J. E. F., Martindale: The Extra Pharmacopoeia (1989) 1246.
2 Abraham, J. S., Bentley, F. R., Gavrison, R. N., and Cryer, H. M., Transplant. Proc. *23* (1991) 356.
3 Moore, J., J. Am. Coll. Toxic. *3* (1984) 1.
4 Abraham, J. S., Bentley, F. R., Gavrison, R. N., and Cryer, H. M., Transplantation *52* (1991) 101.

5 Fogo, A., Hellings, S. E., Inagami, T., and Kon, V., Kidney Int. *42* (1992) 770.
6 Gaudy, J. H., Sicard, J. F., Lhoste, F., and Boiter, J. F., Can. J. Anaesth. *34* (1987) 122.
7 Hunter, S. C., and Thomson, D. S., Br. J. Anaesth. *54* (1982) 231P.
8 Huttel, M. S., Schou-Olesen, A., and Stoffersen, E., Br. J. Anaesth. *52* (1980) 77.
9 Mehring, N., Neumann, K. H., Rahn, K.-H., and Zidek, W., Nephron *60* (1992) 474.
10 Moneret-Vautrin, D. A., Laxenaire, M. C., and Viry-Babel, F., Br. J. Anaesth. *55* (1983) 469.
11 Thiel, G., Hermle, M., and Brunner, F. P., Clin. Nephrol. *25*(Suppl 1) (1986) 540.
12 Zhao, F. K., Chuang, L. F., Israel, M., and Chuang, R. Y., Biochem. biophys. Res. Commun. *159* (1989) 1359.
13 Alade, S. L., Brown, R. E., and Paquet, A., Pediatrics *77* (1986) 593.
14 Arrowsmith, J. B., Faich, G. A., Tomita, D. K., Kuritsky, J. N., and Rosa, F. W., Pediatrics *83* (1989) 244.
15 Balistreri, W. F., Farrell, M. K., and Bove, K. E., Pediatrics *78* (1986) 503.
16 Bove, K. E., Kosmetatos, N., Wedig, K. E., Frank, D. J., Whitletach, S., Saldivar, J., Haas, J., Bodenstein, C., and Balistreri, W. F., J. Am. med. Assoc. *254* (1985) 2422.
17 Gough, W. B., Zeiler, R. H., Barreca, P., and El Sherif, N. J., Cardiovasc. Pharmac. *4* (1982) 375.
18 Martone, W. J., Williams, W. W., Mortensen, M. L., Gaynes, R. P., White, J. W., Lorch, V., Murphy, D., Sinha, S. N., Frank, D. J., Kosmetatos, N., Bodenstein, C. J., and Roberts, R. J., Pediatrics *78* (1986) 591.
19 Masini, E., Planchenault, J., Pezziardi, F., Gautier, P., and Gagnol, J. P., Agents Actions *16* (1985) 470.
20 Munoz, A., Karila, P., Gallay, P., Zettelmeier, F., Messner, P., Mery, M., and Grolleau, R., Eur. Heart J. *9* (1988) 142.
21 Peeters, B. W., Cheung, K. S., Vossen, J. M., and Coenen, A. M., Brain Res. Bull. *29* (1992) 515.
22 Reyniers, J. P., Machado, E. A., and Farkas, W. R., Prog. clin. biol. Res. *181* (1985) 91.
23 Varma, R. K., Kaushal, R., Junnarkar, A. Y., Thomas, G. P., Naidu, M. U. R., Singh, P. P., and Tripathi, R. M., Arzneimittel Forsch. Drug Res. *35* (1985) 804.
24 Yarış, E., Tuncer, M., and Ilhan, M., Clin. Sci. *83* (1992) 179.
25 Lodge, N. J., J. Pharmac. expl Ther. *271* (1994) 730.
26 Feddersen, C. O., McMurtry, I. F., Henson, P., and Voelkel, N. F., Am. Rev. respir. Dis. *133* (1986) 197.
27 Byfield, R. A., Swane, G. T. G., and Warner, T. J., Br. J. Pharmac. *88* (1986) 436P.
28 Cusma-Pelògia, N., Oliveira, S. F., Nigro, D., de Carvalho, M. H. C., Scivoletto, R., and Fortes, Z. B., J. pharmac. toxic. Meth. *29* (1993) 157.
29 Hiley, C. R., Phoon, C. K. L., and Thomas, G. R., Br. J. Pharmac. *91* (1987) 378P.