Table 1. Effect of pH on [$^3$H]norepinephrine uptake into isolated storage vesicles*

| Tissue | Percent uptake pH 7.4 | 7.0 | 6.6 | 6.2 | 5.7 |
|---|---|---|---|---|---|
| Adrenal | 100 ± 4 | 51 ± 8 | 20 ± 3 | 16 ± 5 | 2 ± 1 |
| Brain | 100 ± 3 | 61 ± 3 | 50 ± 5 | 13 ± 3 | 2 ± 1 |
| Heart | 100 ± 4 | 70 ± 5 | 24 ± 3 | 13 ± 2 | 8 ± 2 |

* Data are means ± S.E.M. of six to fifteen determinations. Uptakes at pH 7.4 were 2760 ± 110 dpm for adrenal, 9298 ± 280 dpm for brain, and 2692 ± 108 dpm for heart preparations.

decrement in uptake; in fact, low pH values do not appear to affect efflux or may even reduce it [20]. The most likely explanation, then, is that the uptake process in all these preparations is dependent upon a similar proton gradient, an explanation which is supported by the nearly complete attenuation of uptake at pH 5.7, as predicted by the chemi-osmotic hypothesis which indicates an intravesicular pH of 5.5 [9, 10].

In summary, catecholamine storage vesicles isolated from rat heart, brain and adrenal medulla display similar pH profiles for uptake of [$^3$H]norepinephrine. These studies support the view that the mechanism by which energy is utilized indirectly to transport catecholamines into storage vesicles is common to the various tissues containing these transmitters.

*Acknowledgements*—Research was supported by USPHS HL-24115. T. A. S. is recipient of Research Scientist Development Award DA-00006 from the National Institute on Drug Abuse. G. E. is recipient of a predoctoral fellowship under USPHS Grant GM-07184.

*Department of Pharmacology*
*Duke University Medical Center*
*Durham, NC 27710, U.S.A.*

GARY EVONIUK
THEODORE A. SLOTKIN*

REFERENCES

1. A. Carlsson, *Handbk exp. Pharmac.* **19**, 529 (1965).
2. A. Philippu, in *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines* (Ed. D. M. Paton), pp. 215–46. Raven Press, New York (1976).
3. T. A. Slotkin and D. L. Bareis, *Pharmacology* **21**, 109 (1980).
4. C. L. Bashford, R. P. Casey, G. K. Radda and G. A. Ritchie, *Neuroscience* **1**, 399 (1976).
5. R. Holz, *Proc. natn. Acad. Sci. U.S.A.* **10**, 5190 (1978).
6. R. Johnson and A. Scarpa, *J. biol. Chem.* **251**, 2189 (1976).
7. L. Toll, C. Gundersen and B. Howard, *Brain Res.* **136**, 59 (1977).
8. L. Toll, *Ph.D. Dissertation*, University of California, Los Angeles (1978).
9. R. Johnson, N. Carlson and A. Scarpa, *J. biol. Chem.* **253**, 1512 (1978).
10. D. Njus and G. Radda *Biochim. biophys. Acta* **463**, 219 (1978).
11. K. J. Angelides, *J. Neurochem.* **35**, 949 (1980).
12. D. L. Bareis and T. A. Slotkin, *J. Neurochem.* **32**, 345 (1979).
13. T. A. Slotkin, F. J. Seidler, W. L. Whitmore, M. Salvaggio and C. Lau, *Molec. Pharmac.* **14**, 868 (1978).
14. T. A. Slotkin, M. Salvaggio, F. J. Seidler and W. L. Whitmore, *Molec. Pharmac.* **15**, 607 (1979).
15. F. J. Seidler, D. F. Kirksey, C. Lau, W. L. Whitmore and T. A. Slotkin, *Life Sci.* **21**, 1075 (1977).
16. T. A. Slotkin, in *Neuropoisons: Their Pathophysiological Actions* (Eds. L. L. Simpson and D. R. Curtis), Vol. 2, pp. 1–60. Plenum Press, New York (1974).
17. T. A. Slotkin and N. Kirshner, *Biochem. Pharmac.* **22**, 205 (1973).
18. T. A. Slotkin, F. J. Seidler, W. L. Whitmore, M. Salvaggio and D. L. Bareis, *Neuroscience* **5**, 753 (1980).
19. R. Tanaka, H. Asaga and M. Takeda, *Brain Res.* **115**, 273 (1976).
20. G. Taugner, *Naunyn-Schmiedeberg's Archs Pharmac.* **274**, 299 (1972).

* Address all correspondence to: Dr. Theodore A. Slotkin, Box 3813, Duke University Medical Center, Durham, NC 27710.

*Biochemical Pharmacology*, Vol. 30, No. 20, pp. 2873–2875, 1981.
Printed in Great Britain.

0006-2952/81/202873-03 $02.00/0


# The mechanism of the blockade by trifluoperazine of some actions of phenylephrine on liver and smooth muscle

(*Received* 16 *April* 1981; *accepted* 19 *May* 1981)

The effects of the α-adrenoreceptor agonist phenylephrine on glucose output, $O_2$ consumption and mitochondrial $Ca^{2+}$ fluxes in perfused rat liver are greatly reduced by the phenothiazine derivative, trifluoperazine [1]. This agent has been reported [2–4] to inhibit the action of calmodulin, a $Ca^{2+}$-binding protein implicated in intracellular $Ca^{2+}$-dependent mechanisms (reviewed in [5–7]). Since many of the responses to α-adrenoreceptor activation in a variety of cells (including hepatocytes: see [8, 9]) can be explained as a consequence of a rise in cytosolic $Ca^{2+}$, the blockade by trifluoperazine of the effects of phenylephrine on perfused rat liver has been considered as possible evidence for the involvement of calmodulin in the actions mediated by α-adrenoreceptors in this tissue [1].

A possible complication is that trifluoperazine is also an α-adrenoreceptor blocking agent, as suggested by experiments with smooth muscle [10, 11] and by measurements of its ability to displace labelled WB4101, a potent α-adrenoreceptor antagonist [12, 13] from binding sites in rat brain [14]. To evaluate this possibility in liver tissue, we have compared the ability of trifluoperazine to block the responses to α-agonists, on the one hand, and to ATP and

angiotensin-II on the other. The latter agents were selected because, though acting through distinct receptors, they are thought to raise the concentration of $Ca^{2+}$ in the cytosol of hepatocytes in the same way as suggested for $\alpha$-agonists [8, 9, 15]. One of the consequences of the rise in $Ca^{2+}$ is that the hepatocytes of most species (including the guinea-pig, but not the rat) lose $K^+$, probably because of the opening of Ca-dependent K-channels in their cell membranes [15, 16]. The $K^+$ loss is easily studied by means of a $K^+$-sensitive electrode placed in the suspension fluid [15, 17], and this can provide a convenient means of assessing the action of '$Ca^{2+}$-mobilising agonists', and the effect of trifluoperazine thereon. We have also examined the selectivity of trifluoperazine by comparing its ability to block the contractile responses of smooth muscle (rat vas deferens) to phenylephrine and bradykinin.

*Materials and methods*

The experiments were done at 37° using either dispersed hepatocytes isolated from male guinea-pigs (Hartley, 250–400 g) or 1.0–1.5 cm portions taken from the mid-regions of the vasa deferentia of adult rats (Sprague–Dawley, 200–300 g). Details of the isolation of hepatocytes, and of the use of a $K^+$-sensitive electrode to study drug-induced movements of $K^+$ between them and their suspension medium, have been described in detail [15, 17], as have the methods employed in the experiments with vasa deferentia [13].

Bradykinin triacetate, the disodium salt of adenosine 5′-triphosphate (ATP), isoleucine$^5$ angiotensin II, and (±)-propranolol hydrochloride were purchased from Sigma (London, U.K.), and (−)-noradrenaline bitartrate and (−)-phenylephrine hydrochloride from Koch-Light Laboratories (Colnbrook, U.K.). We are grateful to Smith, Kline and French Ltd. (Welwyn, U.K.) and to Mead Johnson (Evansville, IN) for gifts of trifluoperazine hydrochloride and of (−)-amidephrine hydrochloride, respectively. Each drug was made up freshly before individual experiments, and propranolol was included in all the physiological solutions either at 2 $\mu$M (vasa deferentia) or 10 $\mu$M (hepatocyte experiments).

*Results*

*Liver cells.* As previously described [15, 17], dispersed guinea-pig hepatocytes lose up to 10% of their $K^+$ content within a minute of exposure to ATP or to $\alpha$-adrenoreceptor agonists such as noradrenaline, phenylephrine and amidephrine. Angiotensin II is also effective (see [18]). We tested the action of trifluoperazine on this response to each of these agonists which were applied at concentrations known from earlier work ([15]; T. M. Cocks and D. H. Jenkinson, unpublished data) to cause approximately half-maximal $K^+$ release.

The main findings are shown in Fig. 1. Preincubation of the cells with trifluoperazine for 1–2 min resulted in a concentration-dependent inhibition of the responses to all three $\alpha$-adrenoreceptor agonists; in contrast, the actions of ATP and angiotensin II were unimpaired even by a concentration of trifluoperazine sufficient to abolish $\alpha$-receptor responses.

*Smooth muscle.* The isolated vas deferens of the rat contracts in response to bradykinin as well as to $\alpha$-adrenoreceptor agonists. Figure 2 shows that trifluoperazine (2 $\mu$M) preferentially inhibits the contraction elicited by phenylephrine.

The dose ratios (i.e. the factors by which the concentration of agonist had to be increased to overcome the effect of the antagonist) observed in the four experiments on which Fig. 2 is based were 79, 95, 158 and 107 (geometric mean 106) when phenylephrine was the agonist, as compared with 0.8, 4.8, 4.8 and 0.6 (geometric mean 1.8) when it was bradykinin.

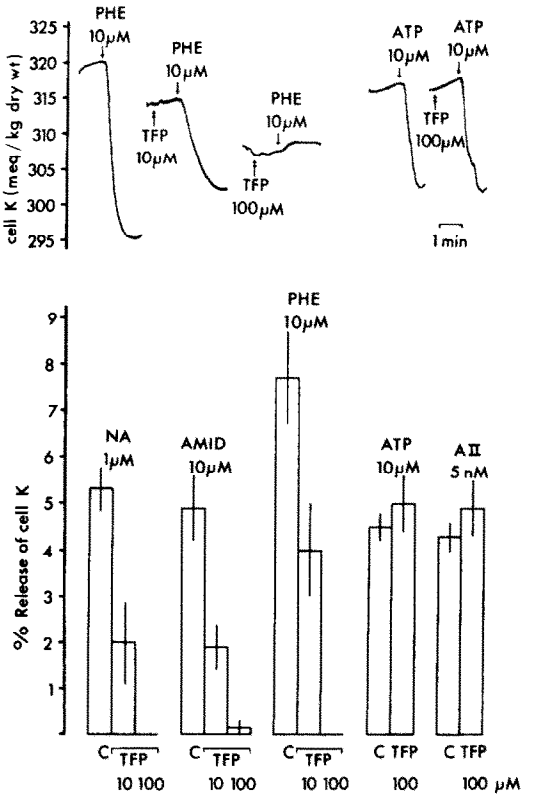


Fig. 1. The effect of trifluoperazine (TFP) on the action of various agonists which cause guinea-pig hepatocytes to lose $K^+$. Upper panel: tracings showing falls in the $K^+$ content of hepatocytes exposed (at arrows) to phenylephrine (PHE) and ATP either in the absence or presence of TFP (added to the suspension 1 min beforehand, at double-headed arrows). Propranolol (10 $\mu$M) was present throughout. The loss of K was measured indirectly by using a $K^+$-sensitive electrode to monitor the corresponding increases in the $K^+$ concentration of the suspension fluid. Lower panel: average $K^+$ losses calculated from such tracings, and expressed as a percentage of the total cell $K^+$ at the time the agonist was applied. TFP (10 or 100 $\mu$M, as indicated) was added to the suspension 1–2 min prior to the various agonists [(−)-noradrenaline, NA; (−)-amidephrine, AMID; (−)-phenylephrine, PHE; adenosine triphosphate, ATP; angiotensin II, AII]. Control responses in the absence of TFP are indicated by C. The column heights give the means of at least 4 values, and the standard errors of the means are shown by vertical lines. TFP at 100 $\mu$M completely abolished the response to NA (1 $\mu$M) and PHE (10 $\mu$M).

*Discussion*

Our results show that trifluoperazine selectively blocks the action of $\alpha$-adrenoreceptor agonists on guinea-pig hepatocytes: the responses to ATP and to angiotensin II are little affected even though both these agents are thought to act by increasing cytosolic $Ca^{2+}$. The simplest explanation, and the one we favour, is that trifluoperazine is an $\alpha$-adrenoreceptor blocking agent (see Introduction). However, other possibilities have not been ruled out. For example, the mechanism whereby receptor activation increases cytosolic $Ca^{2+}$, and so causes $K^+$ channels to open in liver cell membranes, may involve different calmodulins, with only that linked to the $\alpha$-adrenoreceptor being responsive to trifluoperazine.

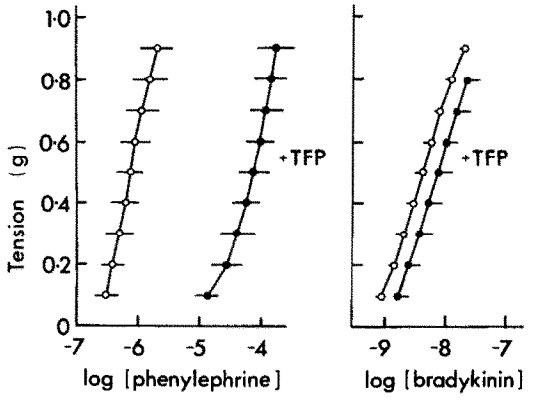


Fig. 2. Comparison of the effects of trifluoperazine on the contractile response of the rat vas deferens to phenylephrine (left) and bradykinin (right). In each of 4 experiments, the mid-portions of the two vasa from a single animal were mounted isometrically in separate organ baths. One preparation was exposed to trifluoperazine (2 $\mu$M) from 30 min before measurements were begun, onwards; the other served as a control. Concentration–response curves for bradykinin and phenylephrine were constructed. From these, the logarithms of the agonist concentrations ($M$) eliciting certain tension increases were read off, and averaged to provide the values shown. The standard errors of the means observed in the 4 experiments are indicated by the horizontal bars. The closed circles were obtained in the presence of trifluoperazine; the open circles are from the control preparations. Propranolol (2 $\mu$M) was present throughout.

That such an explanation is unlikely is supported by our findings with smooth muscle. The greater effectiveness of trifluoperazine against phenylephrine as compared with bradykinin could perhaps be explained by supposing that trifluoperazine selectively interferes with some calmodulin-dependent step or steps between activation of the receptors for $\alpha$-agonists (but not bradykinin) and the ensuing contraction. We think this improbable as there is no reason to suppose that bradykinin and $\alpha$-agonists initiate contraction in fundamentally different ways. It is simpler to suppose, as before, that trifluoperazine is an $\alpha$-adrenoreceptor blocker. If this is so, and if trifluoperazine combines with the receptors with an affinity constant $K$, and has no other actions, then the mean value of the dose ratios we observed suggests, from standard pharmacological considerations, that log $K$ is in the order of 7.7. This is reasonably close to the corresponding figure of 7.3 estimated from radioligand binding experiments [14].

The lower effectiveness of trifluoperazine against $\alpha$-adrenoreceptors in liver as compared with smooth muscle is probably attributable to the remarkable capacity of guinea-pig hepatocytes to inactivate $\alpha$-adrenoreceptor antagonists (G. M. Burgess and D. H. Jenkinson, unpublished observations).

To summarise, we have compared the ability of trifluoperazine to block the action of a range of agonists which initiate potassium loss from guinea-pig hepatocytes by increasing cytosolic calcium. Only the responses to $\alpha$-adrenoreceptor agonists were affected, suggesting that trifluoperazine was acting as an $\alpha$-receptor antagonist rather than as an inhibitor of calmodulin. This was supported by experiments with smooth muscle. Clearly, if the role of calmodulin is to be assessed from the effects of trifluoperazine on intact cells [1, 19], the possibility of other actions and, in particular, of selective receptor blockade, has to be kept in mind. A similar conclusion has been reached by others [20] in a paper published after the present work was completed, and based on somewhat different, though complementary, experiments with rat liver cells.

*Acknowledgements*—We thank Dr. D. G. Haylett for helpful discussion, and the M.R.C. for financial assistance.

*Department of Pharmacology*
*University College London*
*Gower Street*
*London WC1E 6BT, U.K.*

THOMAS M. COCKS
PAULA DILGER
DONALD H. JENKINSON*

REFERENCES

1. P. H. Reinhart, W. M. Taylor and F. L. Bygrave, *FEBS Lett.* **120**, 71 (1980).
2. R. M. Levin and B. Weiss, *Molec. Pharmac.* **13**, 690 (1977).
3. S. Forsén, E. Thulin, T. Drakenburg, J. Krebs and K. B. Séamon, *FEBS Lett.* **117**, 189 (1980).
4. R. E. Klevit, B. A. Levine and R. J. P. Williams, *FEBS Lett.* **123**, (1981).
5. W. Y. Cheung, *Science* **207**, 19 (1980).
6. C. B. Klee, T. H. Crouch and P. G. Richman, *A. Rev. Biochem.* **49**, 489 (1980).
7. A. R. Means and J. R. Dedman, *Nature, Lond.* **285**, 73 (1980).
8. J. W. Putney, *Pharmac. Rev.* **30**, 209 (1978).
9. J. H. Exton, *Am. J. Physiol.* **238**, E3 (1980).
10. I. Takayanagi, *Arzneimittel-Forsch.* **14**, 694 (1964).
11. P. N. Saxena and M. B. L. Johri, *Jap. J. Pharmac.* **23**, 363 (1973).
12. D. R. Mottram and H. Kapur, *J. Pharm. Pharmac.* **27**, 295 (1975).
13. M. Butler and D. H. Jenkinson, *Eur. J. Pharmac.* **52**, 303 (1978).
14. S. J. Peroutka, D. C. U'Prichard, D. A. Greenberg and S. H. Snyder, *Neuropharmacology* **16**, 549 (1977).
15. G. M. Burgess, M. Claret and D. H. Jenkinson, *J. Physiol.* **317**, 67 (1981).
16. D. G. Haylett, *Br. J. Pharmac.* **57**, 158 (1976).
17. G. M. Burgess, M. Claret and D. H. Jenkinson, *Nature, Lond.* **279**, 544 (1979).
18. S. J. Weiss and J. W, Putney, *J. Pharmac. exp. Ther.* **207**, 669 (1978).
19. M. J. Nelson and W. H. Huestis, *Biochim. biophys. Acta* **600**, 398 (1980).
20. P. F. Blackmore, M. F. El-Refai, J.-P. Dehaye, W. G. Strickland, B. P. Hughes and J. H. Exton, *FEBS Lett.* **123**, 245 (1981).

* To whom correspondence should be sent.