clearly be used with caution in experiments involving the neuromuscular junction.

We would like to thank Dr. A. L. Green, Department of Biochemistry, University of Strathclyde, for help with the cholinesterase determinations, and the University of Tabriz, Iran, for financial assistance for one of us (A.G.).

Department of Pharmacology,
University of Strathclyde,
Glasgow G1 1XW, U.K.

A. GANDIHA
I. G. MARSHALL

December 30, 1971

REFERENCES

BAKER, B. R. & GIBSON, R. E. (1971). *J. mednl Chem.*, **14**, 315–322.
BÜLBRING, E. (1946). *Br. J. Pharmac. Chemother.*, **1**, 36–61.
ELLMAN, G. L., COURTNEY, K. D., ANDRES, V. & FEATHERSTONE, R. M. (1961). *Biochem. Pharmac.*, **7**, 88–95.
GINSBORG, B. L. & WARRINER, J. (1960). *Br. J. Pharmac. Chemother.*, **15**, 410–411.
KREBS, H. A. & HENSELEIT, K. (1932). *Hoppe-Seyler's Z. Physiol. Chem.*, **210**, 33–66.
SAMS, W. M., CARROLL, N. V. & CRANTZ, P. L. (1966). *Proc. Soc. exp. Biol. Med.*, **122**, 103–107.
ZETLER, G. & LANGHOF, H.-J. (1971). *Arch. Pharmak.*, **270**, 361–377.

Dimethylsulphoxide effect on myocardial β-adrenoceptors

An unusual spectrum of actions of dimethylsulphoxide (DMSO) on isolated cardiac muscle has been reported. Sams, Carroll & Crantz (1966) noted that DMSO (0·42 and 0·85 M) produced positive inotropism in the guinea-pig isolated atrium-vagus preparation. Karow, Unal & others (1967) and Karow, Carrier & Clower (1968) reported that DMSO (2·1 M in Ringer solution) perfused through the rat isolated heart Langendorff preparation produced precipitous negative inotropism and negative chronotropism. This so called "pseudotoxic effect" (Karow, 1969), was shown to be reversible with DMSO washout. Shlafer & Karow (1971) have further demonstrated with the rat isolated perfused heart that low concentrations (0·14, 0·70 M) of DMSO produced positive inotropism; moderate concentrations (1·41 M) produced a reversible negative inotropism (pseudotoxicity), and high concentrations (2·82 M) produced irreversible negative inotropism (true toxicity); while all concentrations investigated produced reversible negative chronotropism (pseudotoxicity). These observations and normal animal species variability can reconcile the apparently conflicting reports of Offerijns, Freund & Krijnen (1969), Spilker (1970) and of Feuvray & de Leiris (1971) about the action of DMSO (positive or negative inotropism) on isolated cardiac muscle.

To determine the relation existing between the cardiac effects of DMSO and myocardial β-adrenoceptors, the following experiment was done.

Rabbits (approximately 2 kg) were decapitated and hearts immediately removed and placed in Ringer solution (NaCl, 153·9; KCl, 5·4; $CaCl_2$, 2·4; $NaHC0_3$, 16·8; dextrose, 11 mM; distilled water to 1 litre; pH adjusted to 7·4), gassed with 5% carbon dioxide in oxygen. The left atria were isolated, trimmed of peripheral tissue, tied to a Plexiglas holder containing 2 platinum electrodes, and bathed in oxygenated Ringer at 30°. Tension (1·0 g) was placed on each atrium which was stimulated continually throughout the experiment with a square wave of 5 ms duration (1·7 Hz, 5 V). Contractions were recorded by a direct writing oscillograph. To minimize osmotic stress to the muscle, stepwise increments were used to achieve the desired DMSO concentration. All atria were first soaked for at least 15 min in Ringer; some of these were then soaked in Ringer containing 0·14 M DMSO for 15 min. Of the

Table 1. *The effect of DMSO on contractile force (mean ± s.e.) of driven isolated atria with and without drugs acting on β-adrenoceptors.*

DMSO (M)	N	Initial contractile force (g)	Change in contractile force (g) after: Isoprenaline (M) 10^{-9}	10^{-8}	10^{-7}	10^{-6}	Isoprenaline (10^{-7}) + Sotalol (M) 10^{-7}	10^{-6}	10^{-5}	10^{-4}
0·00	14	0·77 ± 0·16	0·01 ± 0·03	0·00	0·75 ± 0·10	0·56 ± 0·20	0·76 ± 0·22	0·60 ± 0·07	0·52 ± 0·09	0·07 ± 0·03
0·14	13	1·07 ± 0·21	0·14 ± 0·13	0·41 ± 0·17	1·65 ± 0·34	1·83 ± 0·33	1·49 ± 0·23	0·77 ± 0·19	0·85 ± 0·23	0·19 ± 0·06
0·42	16	0·75 ± 0·02	0·00	0·26 ± 0·10	1·21 ± 0·18	1·01 ± 0·19	1·14 ± 0·26	0·97 ± 0·13	0·84 ± 0·18	0·19 ± 0·05
0·84	16	0·52 ± 0·08	0·00	0·00	1·30 ± 0·21	1·01 ± 0·14	0·88 ± 0·18	0·64 ± 0·17	0·00	0·00

DMSO-treated atria, some were then soaked in 0·42 M DMSO-Ringer for a further 15 min, and again a proportion were soaked in 0·84 M DMSO-Ringer for 15 min. Thus 4 groups of atria were studied: 14 maintained only in Ringer, 13 ultimately maintained in 0·14, 16 in 0·42 and 16 in 0·84 M DMSO-Ringer.

The responses of all atria to isoprenaline HCl (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) were examined after subjecting the atria to a given concentration of isoprenaline for 10 min, when they were washed 3 times at 5 min intervals before testing the next highest concentration. Atria were always in a solution containing the maintenance concentration of DMSO-Ringer.

The inotropic effect of isoprenaline on the atria was further tested in the presence of sotalol HCl (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M). The atria were soaked in the lower concentration of sotalol for 15 min and then 10^{-7} M isoprenaline was added. After a further 10 min contact period the atria were washed 3 times at 5 min intervals before the next concentration of sotalol was tested similarly.

Soaking the atria in DMSO had no significant ($P > 0.2$) effect on their contractile strength compared to atria soaked in Ringer (Table 1). This is in contrast to the positive inotropism observed by Spilker (1970) in the rabbit isolated left atrium with concentrations as low as 0·07 M DMSO.

Muscle soaked in DMSO gave significantly stronger positive inotropism to isoprenaline than muscle not so treated. DMSO, 0·14 M, enhanced the effect of isoprenaline much more than at 0·42 or 0·84 M, but at these higher concentrations of DMSO, the positive inotropic effect of isoprenaline remained greater than its effect in the absence of DMSO ($P < 0.05$).

Solatol blocked the isoprenaline inotropism significantly better in the presence of DMSO. The antagonistic effect of sotalol on the positive inotropism of 10^{-7} M isoprenaline in Ringer and in DMSO at 0·14 and 0·42 M was significant only at a sotalol concentration of 10^{-4} M ($P \ll 0.001$). The antagonistic effect of sotalol on 10^{-7} M isoprenaline was significant at 10^{-6} M sotalol when 0·84 M DMSO was used ($P < 0.05$). Complete isoprenaline antagonism was achieved with 10^{-5} M sotalol in 0·84 M DMSO.

The diminished mean strength of the atria soaked at the two highest concentrations of DMSO may reflect an incipient pseudotoxic effect, i.e. a negative inotropism and chronotropism, reversible by drug washout.

The general effect of DMSO on cardiac muscle, and, in particular, effects on myocardial β-adrenoceptors are not easy to interpret. DMSO is known to have a wide variety of biological effects which include increasing cell permeability (Csáky & Ho, 1966; Morain, Replogle & Curran, 1966; Franz & Van Bruggen, 1967), altering membrane structure (Norred, Ansel & others, 1970) and stimulating the activity of some enzymes (Rammler, 1967; Perlman & Wolff, 1968; Henderson, Henderson & Johnson, 1969).

Burges, Blackburn & Spilker (1969), using guinea-pig isolated atria, found evidence

that the DMSO effect on cardiac muscle is not mediated through the release of catecholamines or through a direct "catecholamine-like" activity on adrenoceptors. They further observed that DMSO stimulates microsomal Ca^{2+} transport adenosine triphosphatase and adenyl cyclase, and inhibits Na^+, K^+ adenosine triphosphatase. Such alterations in the activity of these enzymes could be responsible for the positive inotropism. The data of Burges & others (1969) indicated that the DMSO effect is probably mediated by microsomal Ca^{2+} uptake and/or Na^+, K^+-ATPase rather than by adenyl cyclase. Additional evidence which implicates these two enzyme systems in the cardiac effects of DMSO has been provided by Melville, Klingner & Shister (1968) who showed that DMSO potentiates the actions of cardiac glycosides *in vivo* without directly influencing glycoside uptake. The present findings with DMSO on β-adrenoceptors in the heart do not resolve the mechanism of the cardiac effect of this substance, however. It may be that the mechanism is concerned more with the interference with the structure and function of water in the cell membrane (Karow & others, 1967; Zetler & Langhof, 1971).

This research was generously supported by grants from the American Heart Association (69–637) and from the United States Public Health Service National Institutes of Health (FR-5365). The author is grateful to Professor R. P. Ahlquist for valuable suggestions and thanks Mrs. A. T. Coppage and Mrs. R. Sims for technical assistance. DMSO was kindly supplied by Mr. R. J. Herschler, Crown Zellerbach Corp.; sotalol was a gift from Dr. G. R. McKinney, Mead Johnson Co.

Departments of Pharmacology and Surgery,
Medical College of Georgia,
Augusta, Georgia 30902 U.S.A.

ARMAND M. KAROW, JR.

December 3, 1972

REFERENCES

BURGES, R. A., BLACKMAN, K. J. & SPILKER, B. A. (1969). *Life Sci.* **8**, 1325–1335.

CSÁKY, T. Z. & HO, P. M. (1966). *Proc. Soc. exp. Biol. Med.*, **122**, 860–865.

FEUVRAY, D. & DE LEIRIS, J. (1971). *Europ. J. Pharmac.*, **16**, 8–13.

FRANZ, T. J. & VAN BRUGGEN, J. T. (1967). *Ann. N.Y. Acad. Sci.*, **141**, 302–309.

HENDERSON, T. R., HENDERSON, R. F. & JOHNSON, G. E. (1969). *Arch. Biochem. Biophys.*, **132**, 342–248.

KAROW, A. M., Jr. (1969). *J. Pharm. Pharmac.*, **21**, 209–223.

KAROW, A. M., Jr., CARRIER, O., Jr. & CLOWER, B. R. (1968). *Ibid.*, **20**, 297–301.

KAROW, A. M., Jr., UNAL, M. O., CARRIER, O., Jr., DANIEL, C. R. & WEBB, W. R. (1967). *Cryobiology*, **3**, 355–340.

MELVILLE, K. I., KLINGNER, B. & SHISTER, H. E. (1968). *Archs int. Pharmacodyn. Thér.*, **174**, 277–293.

MORAIN, W. D., REPLOGLE, C. A. & CURRAN, P. F. (1966). *J. Pharmac. exp. Ther.*, **154**, 298–302.

NORRED, W. P., ANSEL, H. C., ROTH, I. L. & PEIFER, J. J. (1970). *J. pharm. Sci.*, **59**, 618–622.

OFFERIJNS, F. G. J., FREUND, G. E. & KRIJNEN, H. W. (1969). *Nature, Lond.*, **222**, 1174–1175.

PERLMAN, R. L. & WOLFF, J. (1968). *Science, N.Y.*, **160**, 317–319.

RAMMLER, D. H. (1967). *Ann. N.Y. Acad. Sci.*, **141**, 291–299.

SAMS, W. M., Jr., CARROLL, N. V. & CRANTZ, P. L. (1966). *Proc. Soc. exp. Biol. Med.*, **122** 103–107.

SHLAFER, M. & KAROW, A. M., Jr. (1971). *Cryobiology*, **8**, 280–289.

SPILKER, B. (1970). *J. Pharmac. exp. Ther.*, **175**, 361–367.

ZETLER, G. & LANGHOF, H. J. (1971). *Arch. Pharmak*, **270**, 361–377.