This study again emphasizes the danger in placing confidence in a single assay, even when done by a trained person using a well established technique. It also shows the danger of placing confidence in replicate assays performed in parallel by the same analyst, even when the standard deviation for the replicates appears acceptable.

CONCLUSIONS

As shown by the statistical data included in the tables, there is no reason to think that the replacement of permanganate with ceric sulfate changes the mean value of the assay results. Although smaller values for the standard deviations might have been obtained by rejecting some of the results, all data collected have been reported.

REFERENCES

(1) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960.
(2) "The National Formulary," 11th ed., J. B. Lippincott Co., Philadelphia, Pa., 1960.
(3) *Anal. Chem.*, **23**, 1528(1951).

Vasopressin Tachyphylaxis

By P. N. PATIL, A. TYE, and J. W. NELSON

Pretreatment with D-*levo*- ephedrine is known to augment the pressor effect of vasopressin in dogs. It also delayed the development of vasopressin tachyphylaxis in this study. Methoxamine did not produce the same effects. Reserpine augmented the pressor response and arrested the tachyphylaxis. Increasing doses of vasopressin then produced increases in pressor response, an effect which suggests a possible bioassay for vasopressin. No cross tachyphylaxis to pressor effects were observed between vasopressin and angiotensin.

GEILING AND CAMPBELL (1) and Jones and Schlapp (2) have noted the decrease in pressor response to successive doses of posterior pituitary extract. Hogben, *et al.* (3), studied the development of tolerance to posterior pituitary lobe extract in the spinal cat and concluded that it is a function of dose and time interval between doses. By spacing repeated doses at appropriate time intervals, they were able to obtain pressor responses of the same character and magnitude as the one produced by the first injection. Woodbury and Wilks (4) found that in ouabain-treated animals tachyphylaxis to vasopressin developed less readily than in control animals; they suggested that vasopressin tachyphylaxis is a "pseudo-tachyphylaxis." Gardier and Abreu (5) showed that tolerance to vasopressin can be prevented by bilateral carotid sinus denervation and midcervical vagotomy. We investigated the effects of pretreatment with D-*levo*-ephedrine, methoxamine, and reserpine in this study. The sympathomimetic amine, D-*levo*-ephedrine, is a good antagonist of the coronary constriction produced by vasopressin (6); methoxamine is a sympathomimetic amine that produces peripheral effects without cardiotonic effects (7, 8); reserpine blocks carotid and vagal reflexes (9, 10). Cross tachyphylaxis between angiotensin and vasopressin and the effects of renin were also studied.

EXPERIMENTAL

Twenty-five mongrel dogs of each sex, weighing from 7 to 11 Kg., were anesthetized with 35 mg./Kg. of pentobarbital i.p. Both vago-sympathetic nerves were severed. With the usual hemodynamic setup, blood pressure was recorded from the right carotid artery on a kymograph. The trachea was always cannulated. All drugs were dissolved in physiological saline and injected into the femoral vein *via* an indwelling catheter.

Received July 16, 1963, from the College of Pharmacy, The Ohio State University, Columbus.
Accepted for publication August 19, 1963.
Presented to the Scientific Section, A.PH.A., Las Vegas meeting, March 1962.

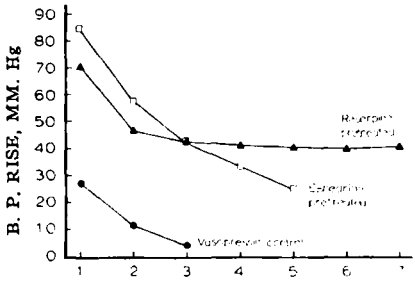


Fig. 1.—The effects of reserpine and D-*levo*-ephedrine pretreatment on vasopressin tachyphylaxis. Each point represents the average blood pressure rise of four to five dogs. The standard errors of the mean varied from 9 to 20% of the average values.

The reserpinized dogs were prepared by administering 0.5 mg./Kg. reserpine i.p. each day for 2 days; the blood pressure was measured as above on the third day. Anesthesia was produced with 15 to 20 mg./Kg. of pentobarbital i.p. in these dogs. Additional pentobarbital was given when necessary to maintain surgical anesthesia.

The following drugs were used: D-*levo*-ephedrine HCl, methoxamine HCl,[1] vasopressin,[2] reserpine phosphate,[3] angiotensin,[4] and hog renin.[5]

RESULTS

In control dogs, vasopressin 0.3 u./Kg. at 30-minute intervals resulted in a progressive reduction of the pressor effect. Tachyphylaxis was practically

[1] Marketed as Vasoxyl by Burroughs Wellcome and Co., Inc., Tuckahoe, N. Y.
[2] Marketed as Pitressin by Parke, Davis and Co., Detroit, Mich.
[3] Marketed as Serpasil by Ciba Pharmaceutical Products, Inc., Summit, N. J.
[4] Marketed as Hypertensin-Ciba by Ciba Pharmaceutical Products, Inc., Summit, N. J. Courtesy of Dr. A. J. Plummer.
[5] Courtesy of Dr. O. M. Helmer, Eli Lilly and Co., Indianapolis, Ind.

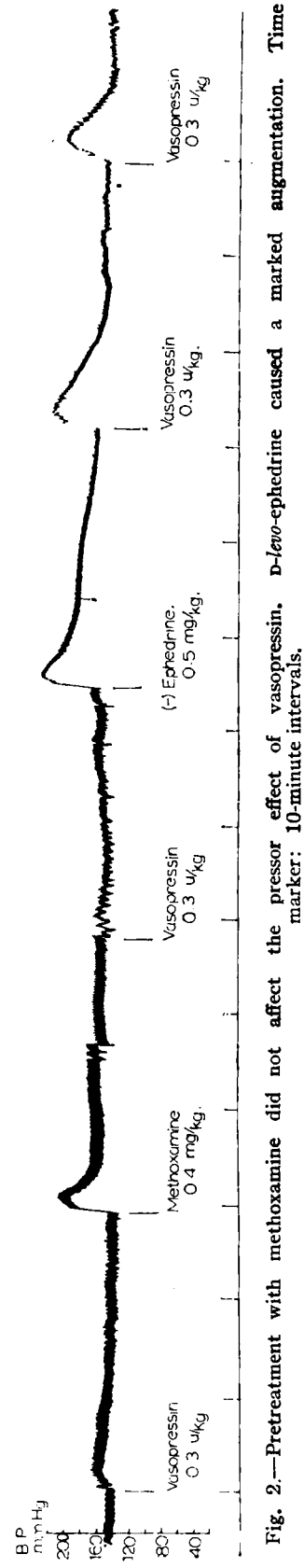


Fig. 2.—Pretreatment with methoxamine did not affect the pressor effect of vasopressin. D-*levo*-ephedrine caused a marked augmentation. Time marker: 10-minute intervals.

complete after two to four such injections (Fig. 1). D-*levo*-ephedrine hydrochloride given in doses of 0.2, 0.5, 1.0, 3.0, and 10.0 mg./Kg., 15 to 30 minutes before the injection of vasopressin, caused a marked augmentation of the pressor response to the latter drug. Doses above 0.5 mg./Kg. of D-*levo*-ephedrine HCl did not cause an increase in this augmentation. Vasopressin repeated at 30-minute intervals in ephedrine pretreated animals still resulted in tachyphylaxis, but the development was slower. A second injection of D-*levo*-ephedrine HCl augmented once more the pressor response to vasopressin. The pressor response to the second dose of D-*levo*-ephedrine HCl was lower than the response to the first dose, indicating that ephedrine tachyphylaxis was unaffected. Figure 1 shows the effect of pretreatment with 0.5 mg./Kg. of D-*levo*-ephedrine HCl on the pressor effects of repeated doses of vasopressin.

Pretreatment with methoxamine in doses of 0.1 to 0.4 mg./Kg., unlike D-*levo*-ephedrine, failed to augment vasopressin's pressor effect (Fig. 2). Pretreatment with reserpine, 0.5 mg./Kg. per day for 2 days, resulted in an augmented pressor effect from vasopressin. A second dose of vasopressin resulted in a somewhat reduced pressor response, but tachyphylaxis was then arrested, and succeeding doses continued to produce the same pressor response. At this stage, increasing doses of vasopressin resulted in increasing pressor response. The results of one such experiment are shown in Fig. 3.

Since cross tachyphylaxis between long acting sympathomimetic amines is well established, we investigated the possibility of cross tachyphylaxis between the octapeptides, vasopressin and angiotensin. Angiotensin itself, 1 mcg./Kg. every 20 minutes for five doses, did not produce tachyphylaxis; intervening doses of vasopressin had no effect on the pressor response to succeeding doses of angiotensin.

Renin is known to produce tachyphylaxis and to reduce or block the pressor response to angiotensin (11, 12). In our experiments, animals given renin 3 G. u./Kg. every 30 minutes for two or three doses exhibited complete tachyphylaxis. Subsequent doses of angiotensin suffered a reduction in pressor effect, but vasopressin effects were essentially unaffected.

DISCUSSION

True tachyphylaxis may be defined as a diminution in response to repeated doses of a drug due to increasing receptor saturation. The suggestion of Woodbury and Wilks (4) that vasopressin produces a pseudo-tachyphylaxis was based on their finding that ouabain offsets the tachyphylactic effect by increasing the force of cardiac contraction. Our results are compatible with this view, for ephedrine, which is known to oppose the coronary vasoconstrictor effects of vasopressin (7), slowed down the development of tachyphylaxis with more regularity than ouabain; while the sympathomimetic amine methoxamine, which has no cardiotonic effects, did not affect the tachyphylaxis. Ephedrine not only slowed down the development of tachyphylaxis but also enhanced the pressor response to vasopressin. This action probably involves not only cardiotonic effects, but also peripheral effects and abolishment of reflexes (13).

Reserpine arrested the development of vasopressin tachyphylaxis, probably by blocking re-

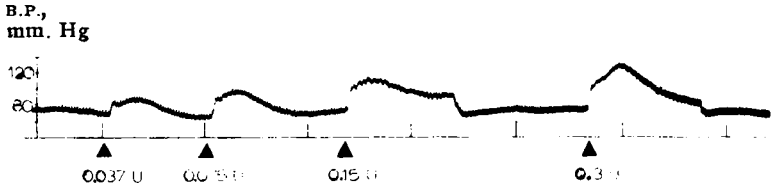


Fig. 3.—Effects of increasing doses of vasopressin after arrest of tachyphylaxis in the reserpinized dog. All doses were in units per kilogram. Time marker: 10-minute intervals.

flexes. Once tachyphylaxis was arrested, dose-response effects were observable. This observation suggests that the use of reserpinized dogs for the bioassay of vasopressin should be investigated.

Our results with angiotensin agreed with those of Page, *et al.* (14). In the pure synthetic form, it was not tachyphylactic and did not affect vasopressin tachyphylaxis. Although both angiotensin and vasopressin are octapeptides, the differences between their structural formulas are sufficient to make it not surprising that there was no cross tachyphylaxis. For the same reason it was expected that, though renin blocked the pressor response to angiotensin, it would not affect the pressor response to vasopressin.

REFERENCES

(1) Geiling, E. M. K., and Campbell, D., *J. Pharmacol. Expll. Therap.*, **29**, 449(1926).
(2) Jones, A. M., and Schlapp, W., *J. Physiol.*, **87**, 144 (1936).
(3) Hogben, L. T., Schlapp, W., and McDonald, A. D., *Quart. J. Expll. Physiol.*, **14**, 301(1924).
(4) Woodbury, R. A., and Wilks, J. W., *Federation Proc.*, **15**, 502(1956).
(5) Gardier, R. W., and Abreu, B. E., *ibid.*, **17**, 370 (1958).
(6) Melville, K. I., and Stehle, R. L., *J. Pharmacol. Expll. Therap.*, **42**, 455(1931).
(7) Goldberg, L. I., Cotten, M., Darby, T. D., and Howell, E. V., *ibid.*, **108**, 177(1953).
(8) Lahti, R. E., Brill, I. C., and McCawley, E. L., *ibid.*, **115**, 268(1955).
(9) Schneider, J. A., *Am. J. Physiol.*, **181**, 64(1955).
(10) Plummer, A. J., Earl, A., Schneider, J. A., Trapold, J., and Barrett, W., *Ann. N. Y. Acad. Sci.*, **59**, 8(1954).
(11) Page, I. H., and Helmer, O. M., *J. Expll. Med.*, **71**, 495 (1940).
(12) Langford, H. G., *Am. J. Physiol.*, **198**, 561(1960).
(13) Lum, B. K. B., and Woodward, J. K., *Federation Proc.*, **21**, 331(1962).
(14) Page, I. H., McCubbin, J. W., Schwarz, H., and Bumpus, F. M., *Circulation Res.*, **5**, 552(1957).

Synthesis of Potential Antineoplastic Agents I

By WILLIAM D. ROLL

Four new derivatives of *o,o'*-diphenamide have been prepared to evaluate their anticarcinogenic activity: *o,o'*-bis(*N*-2-mesyloxyethyl)diphenamide, *o,o'*-bis[*N*-2-(3-chloropropionamido)ethyl]diphenamide, *o,o'*-bis[*N*-2-(3-bromopropionamido)-ethyl]diphenamide, and *o,o'*-bis(*N*-2-mercaptoethyl)diphenamide.

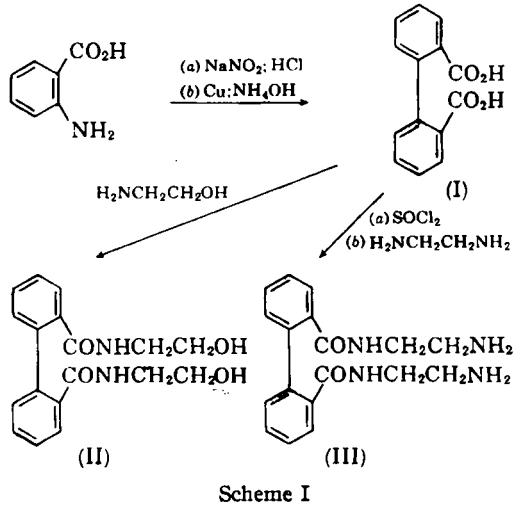
A SERIES OF derivatives of diphenamide was synthesized for the purpose of evaluating their possible antineoplastic activity.

Carbon and co-workers (1) reported that various bis-amides show wide antitumor activity. Baker (2) suggested that the configuration of the "back side" of a molecule may be altered in major ways to give better irreversible bonding and perhaps enhance activity. This report describes the synthesis of some related amides which are structural analogs of diphenamide and which might possibly function as exoalkylating irreversible inhibitors.

The synthetic procedure used for the preparation of these analogs of *o,o'*-diphenamide may be briefly outlined as follows. *o,o'*-Diphenic acid (I), synthesized by the procedure described by Atkinson and Lawler (3), was converted to *o,o'*-bis(*N*-2-hydroxyethyl)diphenamide (II), by a procedure similar to that described by Wenker (4). Treatment of II with methanesulfonyl chloride yielded *o,o'*-bis(*N*-2-mesyloxyethyl)diphenamide (IV).

Treatment of I with thionyl chloride gave *o,o'*-diphenoyl chloride (VII) (5, 6). The acyl halide reacted with ethylene diamine at low temperatures to give the amide *o,o'*-bis(*N*-2-aminoethyl)diphenamide (III). Acylation of the amino analog (III) with 3-chloropropionyl chloride or ethyl 3-chloropropionate and 3-bromopropionyl chloride or methyl 3-bromopropionate gave *o,o'*-bis[*N*-2-(3-chloropropionamido) ethyl]diphenamide and *o,o'*-bis[*N*-2-(3-bromopropionamido)ethyl]diphenamide, compounds V and VI, respectively.

To introduce the mercaptoethyl side chain, diphenic acid (I) was converted to the acyl halide (VII) initially. It was allowed to react with



Scheme I

Received June 3, 1963, from the College of Pharmacy, University of Toledo, Toledo, Ohio.
Accepted for publication August 29, 1963.