Brit. J. Pharmacol. (1959), **14**, 553.

THE USE OF THE GUINEA-PIG ILEUM PREPARATION FOR TESTING THE ACTIVITY OF SUBSTANCES WHICH IMITATE OR ANTAGONIZE THE ACTIONS OF 5-HYDROXYTRYPTAMINE AND TRYPTAMINE

BY

R. B. BARLOW AND I. KHAN

From the Department of Pharmacology, University of Edinburgh

(RECEIVED AUGUST 21, 1959)

On the guinea-pig ileum, 5-hydroxytryptamine appeared to act in the same way as tryptamine on two types of receptor (morphine-sensitive and phenoxybenzamine(Dibenzyline)-sensitive). The actions of analogues of 5-hydroxytryptamine on the phenoxybenzamine-sensitive receptors resembled their actions on the rat uterus, and the actions on the morphine-sensitive receptors slightly resembled those on the rat fundus strip. The guinea-pig ileum preparation, however, did not appear to be more suitable than the rat uterus and rat fundus strip for testing the ability of compounds to imitate or antagonize 5-hydroxytryptamine.

Analogues of 5-hydroxytryptamine are of interest, for not only may they imitate or antagonize the effects of 5-hydroxytryptamine on peripheral tissues but they may have profound effects on the central nervous system. It is difficult to test great numbers of compounds on the central nervous system itself and it is, therefore, desirable that the peripheral tissues chosen for the initial testing of activity should be those on which this activity is most likely to run parallel with that on the central nervous system.

Costa (1956) reported that tranquillizers, such as azacyclonol (Frenquel), chlorpromazine, and reserpine, antagonized the actions of 5-hydroxytryptamine on the rat uterus whereas hallucinogens, such as mescaline and lysergic acid diethylamide, increased the sensitivity to 5-hydroxytryptamine. Cerletti and Doepfner (1958), however, could not obtain any such facilitation with hallucinogens, and it is doubtful whether results obtained on this preparation yield much information about the possible effects of compounds on the central nervous system.

This paper describes experiments with analogues of 5-hydroxytryptamine on the guinea-pig ileum. This tissue contains two types of receptor sensitive to 5-hydroxytryptamine, referred to as M (morphine-sensitive) and D (phenoxybenzamine(Dibenzyline)-sensitive) by Gaddum and Picarelli (1957). The activity of compounds on the M receptors, which are thought to be located in nervous tissue, was considered more likely to run parallel with that on the central nervous system than was the activity on any other preparation in use at the time (J. H. Gaddum, personal communication).

Gaddum (1953) had shown that, after large doses of either 5-hydroxytryptamine or tryptamine, the guinea-pig ileum became insensitive to both 5-hydroxytryptamine and tryptamine. Because of this Gaddum and Picarelli (1957) assumed that the two drugs acted on the same receptors in the guinea-pig ileum. They referred to the M and D receptors as tryptamine receptors even though their experiments were performed with 5-hydroxytryptamine. Woolley and Shaw (1957) and Barlow and Khan (1959a and b) have found substances which antagonize 5-hydroxytryptamine more than tryptamine, which suggests that there may be separate tryptamine and 5-hydroxytryptamine receptors. It was, therefore, desirable to confirm first that the results obtained with 5-hydroxytryptamine on the guinea-pig ileum could also be obtained with tryptamine. This was found to be true and so a number of analogues have been tested on both the M and the D receptors of the guinea-pig ileum. The results are compared with those obtained on the rat uterus and the rat fundus strip (Barlow and Khan, 1959a and b),

and an attempt has been made to assess the value of the estimates of activity obtained with each preparation.

METHODS

The Isolated Guinea-pig Ileum Preparation.—Terminal pieces of ileum from guinea-pigs (150 to 200 g.) deprived of food overnight were suspended in Tyrode solution at 35° in a 2 ml. bath, through which air was bubbled. The movements of the gut were recorded with a light frontal-writing lever, with 5 × magnification, on a smoked paper.

Comparison of 5-Hydroxytryptamine and Tryptamine. — To see if 5-hydroxytryptamine and tryptamine were acting in the same way on the guinea-pig ileum, the doses for equivalent activity of each drug were determined before and after treatment with morphine (3.5×10^{-6} M), phenoxybenzamine (3.4×10^{-7} M) and lysergic acid diethylamide (3×10^{-8} M); the results are expressed as dose ratios. The concentrations of these antagonists were identical with those used by Gaddum and Picarelli (1957). Acetylcholine and nicotine were used as control drugs. Although it was not necessary in determining the dose ratios to plot the complete dose/response curves for 5-hydroxytryptamine and tryptamine, this was done for both drugs before and after the action of lysergic acid diethylamide and for 5-hydroxytryptamine only before and after the action of phenoxybenzamine.

Investigation of the Analogues.—The compounds used are listed in Table IV. Some (see acknowledgments) were given to us; the others we synthesized (Barlow and Khan, 1959a and b).

Exactly the same procedure was used as in the experiments on the rat uterus and rat fundus strip (Barlow and Khan, 1959a), except that the Tyrode solution contained either 3.5×10^{-6} M morphine or 3×10^{-8} M lysergic acid diethylamide.

Experiments were also done on preparations which had been treated with 3.4×10^{-7} M phenoxybenzamine for 30 min. and then washed and suspended in normal Tyrode solution. After this treatment, reproducible responses could only be obtained with 5-hydroxytryptamine for about 2 hr. Thus in any one experiment it was only possible to compare one compound with 5-hydroxytryptamine. In the presence of morphine and lysergic acid diethylamide, however, it was possible to compare 5-hydroxytryptamine with more than one analogue.

RESULTS

5-Hydroxytryptamine and Tryptamine

Dose Ratios.—The dose ratios of 5-hydroxytryptamine and tryptamine for morphine, phenoxybenzamine, and lysergic acid diethylamide are shown in Table I. The values for 5-hydroxytryptamine and tryptamine agree reasonably well, justifying the use of the term tryptamine receptors by Gaddum and Picarelli (1957).

Phenoxybenzamine-treated tissue was so insensitive to the action of tryptamine that no attempt was made to obtain dose ratios with tryptamine. The dose ratios for phenoxybenzamine and lysergic acid diethylamide are given as ranges because, after the preparation had been treated with these drugs, the dose/response curves to 5-hydroxytryptamine and tryptamine, although parallel with each other, were not parallel with the original curves (Fig. 1). This may explain why our estimates of these dose ratios are higher than those (for 5-hydroxytryptamine) of Gaddum and Picarelli (1957). The doses of agonist producing a 50% response varied from one preparation to another depending upon individual sensitivity. Consequently the values of the dose ratios will not be consistent if the dose/response curves for the agonists in the presence of the

TABLE I

ANTAGONISTIC ACTIVITY OF LYSERGIC ACID DIETHYLAMIDE, PHENOXYBENZAMINE, AND MORPHINE ON THE GUINEA-PIG ILEUM AT 35°

The two limits of the ratios for phenoxybenzamine and lysergic acid diethylamide are taken from the two extremes of the dose/response curves of 5-hydroxytryptamine and tryptamine. — Indicates not tested. For morphine, means with standard errors are given.

Drug	No. of Expts.	Conc. ($\times 10^{-7}$ M)	Dose Ratio at the End of 1 hr. for			
			5-Hydroxytryptamine	Tryptamine	Nicotine	Acetylcholine
Phenoxybenzamine	5	3·4	80–40 90–80 100–10 100–20 100	— — — — —	1 1·5 2 3 2	— — — — —
Lysergic acid diethylamide	5	0·3	40–30 50–35 10–8 10–8 25–15	80–40 40–25 40–25 13 —	1 1 1 1 —	1 1 1 1 1
Morphine	4	35	3±0·5	3±0·5	3·5±0·4	1±0

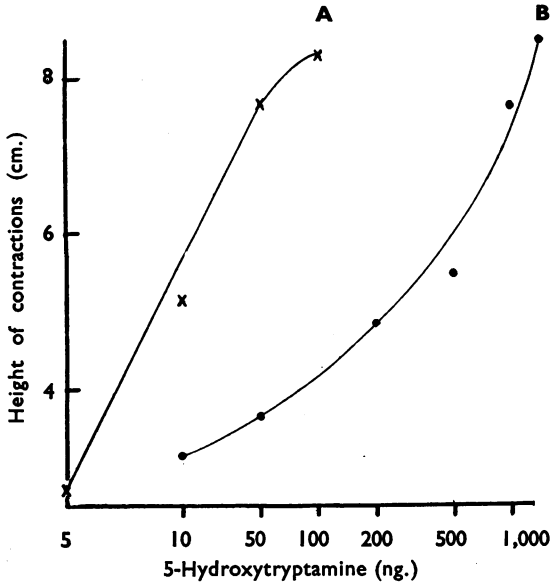


FIG. 1.—Isolated guinea-pig ileum preparation. Log dose/response curves with 5-hydroxytryptamine before (A) and after (B) the action of lysergic acid diethylamide 3×10^{-8} M. Temperature 35°.

antagonists are not parallel with those for the agonists alone.

Effects of Increasing the Concentration of Morphine and Lysergic Acid Diethylamide.—Kosterlitz and Robinson (1955, 1958) showed that increasing the concentration of morphine above 1.7×10^{-7} M did not increase its antagonism of 5-hydroxytryptamine (a concentration 470 times larger, 8×10^{-5} M, gave the same dose ratio), and Gaddum and Picarelli (1957) found that with 3.5×10^{-6} M morphine the M receptors appeared to be more or less completely blocked. Further, it has been shown by Gaddum and Hameed (1954) that raising the concentration of lysergic acid diethylamide from 3×10^{-8} M to 3×10^{-5} M did not increase the antagonism of 5-hydroxytryptamine.

These experiments have been repeated using tryptamine, nicotine, and acetylcholine as well as 5-hydroxytryptamine, and it was found that tryptamine behaved like 5-hydroxytryptamine. The concentration of morphine (3.5×10^{-6} M) produced a maximal antagonism of 5-hydroxytryptamine, tryptamine, and nicotine, but had no effect on responses to acetylcholine. A ten-fold rise in the concentration of morphine did not increase the antagonism of 5-hydroxytryptamine, tryptamine, and nicotine, and had no effect against acetylcholine.

The concentration of lysergic acid diethylamide used (3×10^{-8} M) produced maximum antagonism of 5-hydroxytryptamine and tryptamine, but had no effect against nicotine. This resistance of the effects of nicotine to antagonism by morphine confirms the findings of Kosterlitz and Robinson (1955, 1958).

Effect of Temperature.—In the first experiments with the guinea-pig ileum, the temperature was 35° and there was considerable spontaneous activity of the tissue. In order to reduce this, the temperature was lowered to 28°, and it was then found that the effects of morphine were very feeble. The dose ratios using 3.5×10^{-6} M

TABLE II

ANTAGONISTIC ACTIVITY OF SOME INDOLE DERIVATIVES ON THE M AND D RECEPTORS IN THE GUINEA-PIG ILEUM AT 35°

5-HT, 5-hydroxytryptamine; N, nicotine; H, histamine; Ach, acetylcholine, and — = not tested. Antagonism of 5-hydroxytryptamine by *N'N'*-dimethyltryptamine and 5-benzyloxy-*N'N'*-dimethyltryptamine was maximal at the end of 1 hr., but their antagonism of histamine was maximal within 15 to 20 min. Antagonism of nicotine by 5-benzyloxy-*N'N'*-dimethyltryptamine was maximal only after at least 1 hr. On the phenoxybenzamine-treated preparation the antagonists were only allowed to act for 15 min., but no effects were observed.

Serial No.	Drug	Conc. ($\times 10^{-7}$ M)	Dose Ratios at the End of 1 hr.						
			In Presence of Morphine (D Receptor)					After Phenoxybenzamine Treatment (M Receptor)	
			No. of Expts.	5-HT	N	H	Ach	No. of Expts.	5-HT
8	5-Benzyloxygramine	3·6	2	14 9	1 1	1 1	— —	1	1 —
		7·2	1	15	3	1	—		—
7	5-Benzyloxy-*N'N'*-dimethyltryptamine	62	3	33 36 110	130 100 66	20 3 7	1 1 1	1	1 — —
6	*N'N'*-Dimethyltryptamine	66	3	14 20 14	1 1 0·8	17 20 30	— — —	1	1 — —

TABLE III

ANTAGONISTIC ACTIVITY OF SOME INDOLE DERIVATIVES ON THE GUINEA-PIG ILEUM AT 35° IN THE PRESENCE OF MORPHINE (D RECEPTORS)

Drug ratios are calculated on a molar basis, and given as means with standard errors.

Serial No.	Drug	Conc. ($\times 10^{-7}$ M)	No. of Expts.	Drug Ratio
8	5-Benzyloxygramine	3·6	2	0·92±0·28
		7·2	1	1·00
7	5-Benzyloxy-*N'N'*-dimethyltryptamine	60	3	0·33±0·1
6	*N'N'*-Dimethyltryptamine	66	3	0·055±0·002

morphine were 1.6±0.4 for 5-hydroxytryptamine and 1.4±0.4 for tryptamine (means of six experiments with standard errors), whereas at 35° they were 3.0±0.5 and 3.0±0.5 (Table I). This result was surprising. Kosterlitz and Robinson (1957, 1958) and Innes, Kosterlitz and Robinson (1957) found that morphine, atropine, and lowering the bath temperature reduced contractions of the longitudinal muscle of the guinea-pig ileum caused by 5-hydroxytryptamine. We had, therefore, expected that the responses to 5-hydroxytryptamine would be reduced even further by morphine when the bath temperature was lowered. That they were not suggested that lowering the temperature also reduced the sensitivity of the M receptors.

Investigation of the Analogues of 5-Hydroxytryptamine

Antagonistic Activity.—In the presence of morphine only three of the compounds examined (that is, on the D receptors) antagonized 5-hydroxytryptamine, and on the phenoxybenzamine-treated tissue (on the M receptors) none had any antagonistic activity (Tables II and III).

As we had hoped that tranquillizers would antagonize 5-hydroxytryptamine on the M receptors, we also tested reserpine in concentrations up to 10^{-6} M and found that it was inactive against 5-hydroxytryptamine. Higher concentrations reduced the responses to 5-hydroxytryptamine and also those to histamine and nicotine.

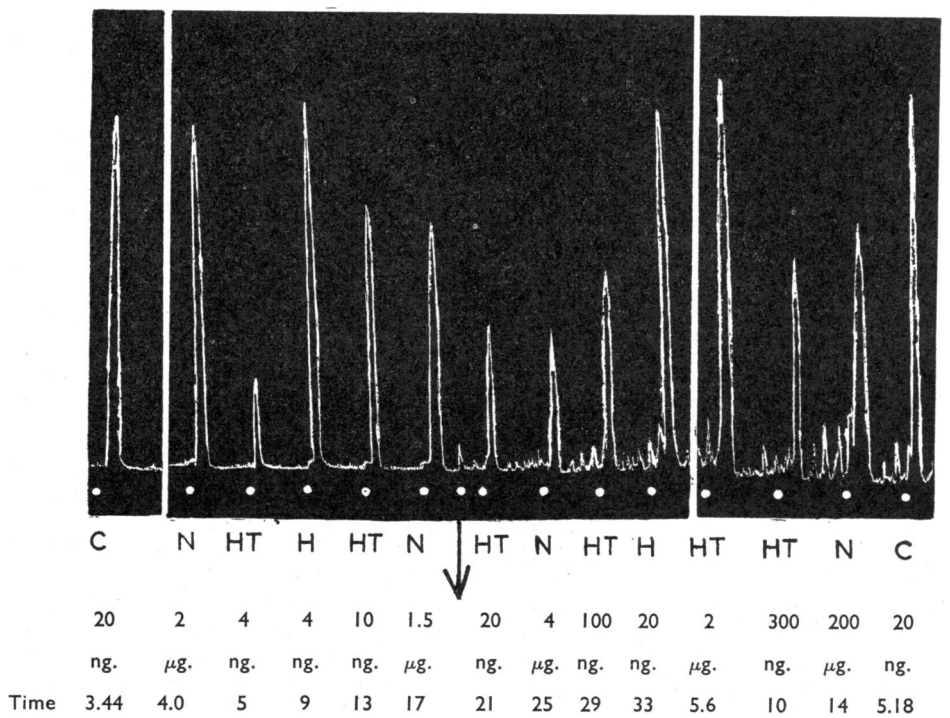


FIG. 2.—Isolated guinea-pig ileum preparation. Antagonistic effect of the analogue, 5-benzyloxy-*N'N'*-dimethyltryptamine (6.2×10^{-6} M at the arrow), on the D receptors in the presence of morphine 3.5×10^{-6} M. Responses are shown to 5-hydroxytryptamine (HT), nicotine (N), histamine (H), and carbachol (C) before and after the addition of the analogue to the bath at 4.19 p.m. At the end of 1 hr. the dose ratios were 33 for 5-hydroxytryptamine, 132 for nicotine, 20 for histamine, and 1 for carbachol. The last response to histamine is not shown in the tracing.

Two compounds tested on the D receptors had interesting side effects. First, 5-benzyloxy-*N'N'*-dimethyltryptamine (*7*) antagonized the action of nicotine (Fig. 2), which action has been termed "morphine insensitive" by Kosterlitz and Robinson (1958). Secondly, *N'N'*-dimethyltryptamine (*6*) and, to a lesser extent, 5-benzyloxy-*N'N'*-dimethyltryptamine (*7*) antagonized the action of histamine.

Stimulant Activity.—The equipotent molar ratios of the compounds tested are shown in Table IV. In the presence of morphine, contractions produced by all the stimulant compounds including 5-hydroxytryptamine and tryptamine were complete within 30 to 45 sec. Tryptamine, 5-hydroxytryptamine, and 5-hydroxy-α-methyltryptamine (*2*) all appeared to be washed out easily, but after the other compounds a slightly longer time (usually 2 min.) was required before the tissue relaxed. In concentrations used for determining the dose/response curve, the drugs produced only a single contraction of the muscle. A high concentration of *N'N'*-dimethyltryptamine (*6*) caused slight depression (lasting about 5 min.) of the control responses to 5-hydroxytryptamine, but after the other compounds the control responses were not disturbed.

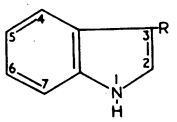
The dose/response curve of *N'N'*-dimethyltryptamine (*6*), though parallel to those of 5-hydroxytryptamine and tryptamine in low concentrations, flattened out in higher concentrations. The curves of the other compounds were parallel to those of 5-hydroxytryptamine and tryptamine over the whole range. Of the compounds tested, 5-benzyloxygramine (*8*), 5-benzyloxy-*N'N'*-dimethyltryptamine (*7*) and α-ethyltryptamine (*5*) were inactive. α-Methyltryptamine (*4*) and *N'N'*-dipropyltryptamine (*3*) were about as active as tryptamine. 5-Hydroxy-α-methyltryptamine (*2*), the most active compound, was about half as active as 5-hydroxytryptamine.

On the preparation treated with phenoxybenzamine, 5-hydroxy-α-methyltryptamine (*2*) was the only compound which was active enough for a dose/response curve to be obtained. This was parallel to that of 5-hydroxytryptamine. The other compounds tested, even in very high concentrations, produced only small effects.

Dose/response curves were obtained with a few compounds in the presence of lysergic acid

TABLE IV

STIMULANT ACTIVITY ON THE GUINEA-PIG ILEUM



Numerals in parenthesis indicate the number of experiments; when more than two were done, means and standard errors are given. Results marked with an asterisk were obtained with threshold responses; the dose/response curves could not be plotted. † Indicates that the top of the dose/response curve was not parallel to that of 5-hydroxytryptamine.

Serial No.	Ring Substituent	Side-chain (R) (Trivial Name)	Stimulant Activity (Equipotent Molar Ratios)		
			D Receptors, 35°	M Receptors, 35°	
			In the Presence of Morphine ($3{\cdot}5\times10^{-6}$M)	Phenoxybenzamine-treated Tissue	In the Presence of LSD (3×10^{-8}M)
1	5-OH	$-CH_2-CH_2-N(C_3H_7)_2$ (5-Hydroxy-*N'N'*-dipropyltryptamine)	12, 20 (2)	120±0 (2)*	12·5, 25 (2)†
2	5-OH	$-CH_2-CH(CH_3)-NH_2$ (5-Hydroxy-α-methyltryptamine)	2·3±0·03 (3)	4, 16 (2)	3·2, 8 (2)
3	—	$-CH_2-CH_2-N(C_3H_7)_2$ (*N'N'*-Dipropyltryptamine)	150±53 (4)	148, 296 (2)*	10±3·8 (4)†
4	—	$-CH_2-CH(CH_3)-NH_2$ (α-Methyltryptamine)	370±110 (3)	>200, 640 (2)*	390±125 (3)*
5	—	$-CH_2-CH(C_2H_5)-NH_2$ (α-Ethyltryptamine)	>20,000 (1)	>200 (1)	—
6	—	$-CH_2-CH_2-N(CH_3)_2$ (*N'N'*-Dimethyltryptamine)	130±40 (3)†	100 (1)*	—
7	5-OC_7H_7	$-CH_2-CH_2-N(CH_3)_2$ (5-Benzyloxy-*N'N'*-dimethyltryptamine)	5,000, >2,000 (2)*	>200 (1)	
8	5-OC_7H_7	$-CH_2-N(CH_3)_2$ (5-Benzyloxygramine)	>5,000 (1)	>200 (1)	
9	5-OH	$-CH_2-CH_2-NH_2$ (5-Hydroxytryptamine)	1	1	1
10	—	$-CH_2-CH_2-NH_2$ (Tryptamine)	162±40 (8)	360, 720 (2)*	257±30 (5)

diethylamide. The equipotent molar ratios are shown in Table IV. 5-Hydroxy-α-methyltryptamine (*2*) was again the most active compound, and its dose/response curve was parallel to those of 5-hydroxytryptamine and tryptamine. *N'N'*-Dipropyltryptamine and 5-hydroxy-*N'N'*-dipropyltryptamine (*3* and *1*) were the next most active, being stronger than tryptamine, but their dose/response curves flattened out at high concentrations. The action of these latter compounds could only be shown on the M receptors when the D receptors were blocked by lysergic acid diethylamide. Phenoxybenzamine left the tissue so insensitive (even to 5-hydroxytryptamine) that responses to the less active compounds could only be obtained with very high concentrations indeed.

One of the most striking features of Table IV is the high activity of *N'N'*-dipropyltryptamine (*3*) on the M receptors.

Effect of Higher Concentrations.—With this preparation, either in the presence of morphine or on the phenoxybenzamine-treated tissue, there was no indication with any of the compounds of the production of repeated contractions such as was seen with the rat uterus (Barlow and Khan, 1959a and b). A multiple response was sometimes seen, but the tissue rapidly relaxed in spite of the presence of the drug. The preparation was then equally insensitive to 5-hydroxytryptamine and tryptamine (even after the drug was washed out), but the control responses to nicotine, acetylcholine, and histamine were unaffected. This behaviour was also seen when lower concentrations (below those producing a maximal response) were left in the bath for a long time (more than 5 to 10 min.).

Discussion

The results of the dose/ratio experiments confirm that tryptamine and 5-hydroxytryptamine act in the same way on the "M" and "D" receptors of the guinea-pig ileum.

Estimates of the stimulant activity of analogues of 5-hydroxytryptamine on the D receptors (in the presence of morphine) resemble those on the rat uterus but not those on the rat fundus strip, on which α-methyltryptamine (*4*) and *N'N'*-dipropyltryptamine (*3*) were much more active than tryptamine (Barlow and Khan, 1959a and b). Estimates of the antagonistic activity on the D receptors also resemble those on the rat uterus.

The high activity of *N'N'*-dipropyltryptamine (*3*) on the M receptors is similar to that on the rat fundus strip, but the dose/response curve of this compound does not flatten out at high concentrations for the rat fundus strip as it does for the M receptors. None of the compounds, not even reserpine, antagonized the effects of 5-hydroxytryptamine on the M receptors, and this test does not appear to indicate any central properties the compounds may have.

The guinea-pig ileum does not seem to have any distinct advantages over the rat uterus and rat fundus strip used as a combined test for ability to imitate or antagonize the actions of 5-hydroxytryptamine. Although the guinea-pig ileum in the presence of morphine has the advantage over the rat uterus that it reveals antihistamine or antinicotine activity as well as ability to imitate or antagonize 5-hydroxytryptamine, this is offset by the disadvantage of having morphine continually present and of working with an extremely complicated tissue.

We wish to thank Professor J. H. Gaddum most sincerely for suggesting this work to us, and Professor W. L. M. Perry for helpful comments on the manuscript. We thank Dr. H. C. Carrington, of the Pharmaceutical Division of Imperial Chemical Industries, Ltd., for the 5-hydroxy-α-methyltryptamine, α-methyltryptamine, and α-ethyltryptamine. This work was done during the tenure by one of us (I. K.) of a Pakistan Government Scholarship.

References

Barlow, R. B., and Khan, I. (1959a). *Brit. J. Pharmacol.*, **14**, 99.
—— —— (1959b). Ibid., **14**, 265.
Cerletti, A., and Doepfner, W. (1958). *J. Pharmacol.*, **122**, 124.
Costa, E. (1956). *Proc. Soc. exp. Biol., N.Y.*, **91**, 39.
Gaddum, J. H. (1953). *J. Physiol. (Lond.)*, **119**, 363.
—— and Hameed, K. A. (1954). *Brit. J. Pharmacol.*, **9**, 240.
—— and Picarelli, Z. P. (1957). Ibid., **12**, 323.
Innes, I. R., Kosterlitz, H. W., and Robinson, J. A. (1957). *J. Physiol. (Lond.)*, **137**, 396.
Kosterlitz, H. W., and Robinson, J. A. (1955). Ibid., **129**, 18*P*.
—— —— (1957). Ibid., **136**, 249.
—— —— (1958). *Brit. J. Pharmacol.*, **13**, 296.
Woolley, D. W., and Shaw, E. (1957). *J. Pharmacol.*, **121**, 13.