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Pharmacological Receptors of the Cerebral Arteries of the Goat[1]

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Abstract. 5-Hydroxytryptamine (5-HT), norepinephrine (NE), histamine (H) and potassium (K^+) chloride induce dose-dependent changes in tension of the isolated middle cerebral artery of the goat. Vasopressin produces highly variable responses followed by tachyphylaxis; angiotensin II is ineffective over a wide dose range. The order of potencies of these vasoactive agents is 5-HT>NE>H>K^+. With regard to their ability to induce maximal contractile responses, the order is: H>5-HT, K^+>NE.

Lysergic acid diethylamide (LSD) antagonizes the actions of 5-HT in a manner which progresses from surmountability to unsurmountability of the blockade depending on the concentration of LSD. The blockade exerted by LSD is reversed by washing. Phentolamine and diphenhydramine competitively antagonize the actions of NE and H, respectively. The potency of phentolamine and diphenhydramine in the cerebral arteries of the goat is similar to that determined in different tissues obtained from a variety of animal species. It is concluded that the cerebral arteries of the goat possess receptors for biogenic amines, the most effective of which is 5-HT; receptors for vasoactive peptides are ill defined.

Several *in vitro* studies have demonstrated that the cerebral blood vessels of various animal species have the ability to develop tension or to increase resistance to flow in response to vasoactive amines [2, 15, 18, 23]. The analysis of the pharmacological receptors involved in these responses has been carried out by studying the effects of specific antagonists upon responses to single doses of agonists [18]. This approach offers qualitative information regarding the type of receptors present in the cerebral blood vessels, but it

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leaves unexamined the nature of the drug-receptor interactions. Data from such quantitative studies on drug-receptor interactions are necessary to compare the properties of the receptors present in the cerebral vasculature with those in other tissues [1].

The present study was undertaken to examine the type of pharmacological receptors present in the isolated cerebral arteries of the goat, to analyse the nature of the drug-receptor interactions using classical pharmacological methodology. This involves determining the effects of relatively specific antagonists upon complete agonist dose-response curves. The goat was selected as the experimental animal because it was considered of interest to extend *in vivo* observations made in our laboratory in the unanesthetized goat which indicated that sympathetic nerve stimulation or intraarterial administration of tyramine induced changes in cerebral blood flow. Since such changes are reduced by α-adrenergic receptor blocking agents they are probably the result of activation of α-adrenergic receptors present in the cerebral vasculature [11, 12], as are the responses of exogenous catecholamines [13]. A preliminary report of the present work has been presented elsewhere [25].

Methods

Goat brains were obtained from the local slaughter house within 1 h of the death of the animal. The middle cerebral artery was carefully dissected and cut into cylindrical segments of 5 mm length. The approximate outside diameter of the artery was 500 μm. The cylinder was set up for isometric recording in an organ bath according to the method described by NIELSEN and OWMAN [18]. Briefly, the method consists of passing two fine stainless steel pins through the lumen of the vascular segment. One pin is fixed to the organ bath wall while the other is connected to strain gauge for isometric recording. The latter pin is parallel to the former and movable permitting the application of resting tension at right angles to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3, a Statham Micro-Scale Accessory U15, and a Beckman Type RS recorder. A resting tension of 1 g was applied to the tissue and readjusted every 15 min during an initial 1-hour period of equilibration.

The organ bath contained 3 ml of Krebs-Henseleit solution continuosly bubbled with 95% oxygen and 5% carbon dioxide, pH 7.3–7.4, and kept at 37°C. The composition of the Krebs-Henseleit solution was (mM): NaCl, 115; KCl, 4.6; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, $7H_2O$, 1.2; $NaHCO_3$, 2.5; glucose, 11.1. Ethylenediamine tetraacetic acid (EDTA), 3×10^{-5} M, was added to prevent oxidation of unstable substances. Drugs were dissolved in saline solution; the total volume added to the organ bath did not exceed 0.4 ml.

Interactions between either 5-hydroxytryptamine (5-HT) or potassium chloride (KCl) and lysergic acid diethylamide (LSD). Cummulative dose-response curves for 5-HT were determined in the same vascular segment in the absence, in the presence and after removal

of LSD (6.3×10^{-9} M). The antagonist was added to the organ bath 10 min before the agonist. Increasing concentrations of LSD (2.1×10^{-9}, 6.3×10^{-9} and 2.1×10^{-8} M) were tested for their ability to antagonize the responses to 5-HT according to the following experimental design. Two vascular segments from the same animal were repeatedly challenged with low doses of 5-HT until a constant level of sensitivity was attained. Then, progressively increasing concentrations of LSD were added to one segment and its sensitivity to 5-HT tested after 10 min incubation with the antagonist. In order to obtain responses of equal magnitude as those elicited prior to the addition of the antagonist, higher doses of 5-HT were necessary. The other segment served as a control for changes in sensitivity with time. The control tissue was challenged with 5-HT at the same time intervals at which this agonist was added to the experimental segment exposed to LSD. As sensitivity to 5-HT did not change with time as indicated by superimposition of the dose-response lines determined during the course of an experiment no correction was made to the effects induced by LSD on the 5-HT dose-response relationship.

Dose-response relationships for KCl were established in vascular segments from different animals either in the absence or in the presence of LSD (6.3×10^{-9} M) which had been added 10 min previously to the bathing solution.

Norepinephrine (NE) – phentolamine (Ph) interactions. Cummulative dose-response curves for NE were determined in vascular tissues from different animals either in the absence or in the presence of Ph 10^{-6} M. The antagonist was added 10 min before the agonist. To minimize oxidation of NE, stock and working solutions of this amine were prepared in saline-containing ascorbic acid 0.01% (w/v).

Geometric mean ED_{50} values were obtained in the manner described by FLEMING *et al.* [4]. The ratio of equieffective doses in the absence and in the presence of antagonist, i.e. the dose ratio (x), was determined at the ED_{50} level. The apparent dissociation constant (Kb) of the antagonist-receptor complex was calculated by means of the formula

$$x-1 = \frac{[B]}{Kb},$$

where [B] refers to the molar concentration of antagonist [7]. This formula is applicable to an equilibrium competitive type of agonist-antagonist interaction. The negative logarithm of Kb is considered equivalent to the pA_2 value, and it was, therefore, used for comparison with pA_2 values reported in the literature for the corresponding antagonists [1].

Histamine (H) – diphenhydramine (D) interactions. Vascular tissues from different animals were used to determine cummulative dose-response curves for histamine either in the absence or in the presence of diphenhydramine (3.4×10^{-7} M), which had been added 10 min previously to the organ bath. For calculating the Kb of the diphenhydramine-receptor complex, a procedure similar to the one described above for the NE-Ph interaction was followed.

Normalizing procedures used for constructing dose-response curves. The results of those experiments in which the control and experimental data were determined in tissues from different animals, such as in the study of the interactions between K^+ and LSD, NE and Ph, and H and D, were normalized on the basis of the percent of the maximum response attained in each vascular segment. The size of the horizontal shifts of the curves was assessed at the ED_{50} level.

In the case of the interaction between 5-HT and LSD, the experiments were carried out in the same vascular segment in the absence, in the presence and after removal of LSD. These results were normalized in terms of percent of the maximum response elicited by 5-HT before adding LSD.

Drugs used were: 5-hydroxytryptamine creatinine sulfate complex (Sigma), l-norepinephrine hydrochloride (Sigma), histamine diphosphate (Sigma), angiotensin amide (Hypertensin, Ciba), lysine-vasopressin (Sigma), lysergic acid diethylamide (Sandoz), phentolamine methanesulfonate (Regitin, Ciba) and diphenhydramine (Benadryl, Parke-Davis). Drug concentrations are expressed in final molar concentration in the bath. The statistical analysis was done by means of the Student's t test [21]; a probability value of less than 5% was considered significant.

Results

Relative Activity of Biogenic Amines and Potassium Chloride

The mean dose-response curves for several agents determined in the middle cerebral artery of the goat are illustrated in figure 1. Responses were expressed as a percent of the maximum tension development achieved with each agent. The relative order of potencies was 5-hydroxytryptamine (5 HT), 1-norepinephrine (NE), histamine (H), potassium chloride (KCL). The geometric mean ED_{50} values for these agents, as well as the maximal responses induced, are given in table I. Notice that the relative order of potencies, expressed quantitatively in terms of the ED_{50} values, differs from the relative order established by the maximal tension developed. Histamine induced the highest change in tension, followed by 5-HT and KCl; the least effective agent in this regard was norepinephrine.

Effects of Lysergic Acid Diethylamide on the Responses to 5-Hydroxytryptamine and Potassium

Complete dose-response curves for 5 HT were obtained in the same vascular segment prior to and in the presence of LSD, and after its removal by repeated wash-outs every 10 min for 30–40 min. The results of these experiments are illustrated in figure 2. LSD (6.3×10^{-9} M) shifted the control curve for 5-HT to the right depressing both its slope and maximum; after removing LSD the tissue regained almost completely its original sensitivity to 5-HT, as indicated by the closeness of the curves determined prior to and after wash-out of the antagonist. Furthermore, the maximum response to 5-HT which had been depressed to about 50% of the control by LSD, was restored to control levels after the removal of the antagonist (table II).

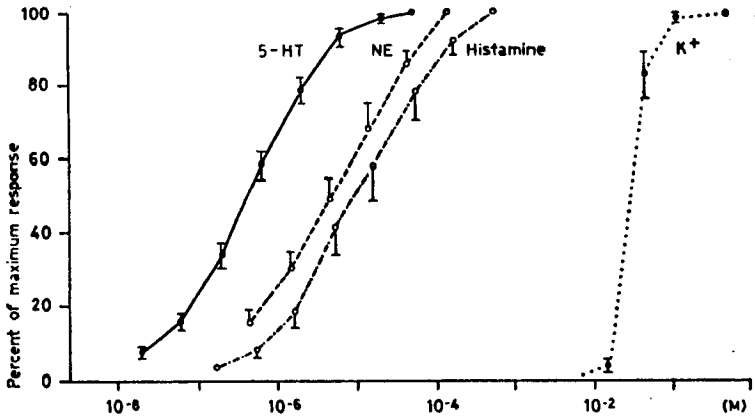

Fig. 1. Dose-response curves for 5-hydroxytryptamine, norepinephrine, histamine and potassium chloride. Vertical bars represent standard errors of the means. Number of experiments are given in table I.

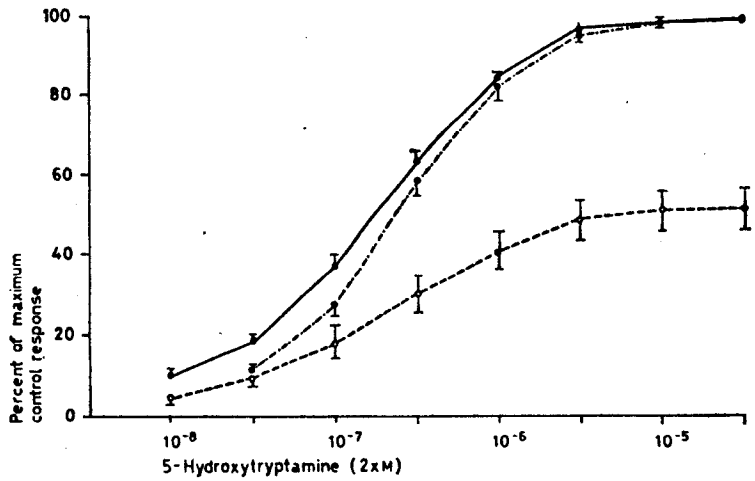

Fig. 2. Dose-response curves for 5-hydroxytryptamine determined in the absence (•—•), in the presence (○---○) and after removal (•-·-·-•) of lysergic acid diethylamide (LSD) 6.3×10^{-9} M. Vertical bars represent standard errors of the means. Number of experiments, six.

Table I. Geometric mean ED_{50} and maximal responses of 5-hydroxytryptamine, norepinephrine, histamine and potassium chloride determined in the isolated middle cerebral artery of the goat

Agonist	Cerebral arteries		n
	geometric mean ED_{50} (95% confidence interval)	maximum response g ± SE	
5-HT	$4.5\ (3.1–6.4) \times 10^{-7}$ M	1.140 ± 0.116	6
NE	$3.5\ (1.6–7.7) \times 10^{-6}$ M	0.345 ± 0.032	14
H	$1.1\ (0.2–5.1) \times 10^{-5}$ M	2.370 ± 0.289	4
K^+	$2.9\ (2.5–3.3) \times 10^{-2}$ M	1.340 ± 0.093	7

n = Number of experiments.

Table II. Maximal responses induced by 5-hydroxytryptamine and potassium chloride in the isolated middle cerebral artery of the goat in the absence, in the presence and after removal of lysergic acid diethylamide (6.3×10^{-9} M)

Agonist	Cerebral arteries maximum response g ± SE		
	control	LSD 6.3×10^{-9} M	after LSD
5-HT	1.140 ± 0.116 (n = 6)	0.593 ± 0.064[1] (n = 6)	1.160 ± 0.221 (n = 6)
K	1.340 ± 0.093 (n = 7)	1.275 ± 0.237 (n = 4)	

[1] $p < 0.005$, paired samples analysis.

This particular concentration of LSD (6.3×10^{-9} M) did not affect the dose-response curves for potassium chloride (fig. 3). The maximum response attained with KCl was not significantly altered by LSD (table II).

The effects of several concentrations of LSD, were tested against the responses induced by 5-HT. The results of a representative experiment are shown in figure 4. Notice that LSD 2.1×10^{-9} M produces a parallel shift of the dose-response curve, but higher concentrations of LSD progressively depress the slope of the dose-response segments. With LSD 2.1×10^{-8} M a small development of tension was observed, averaging 40–60 mg, which subsided before the addition of 5-HT. The ratio of equieffective doses (×) was deter-

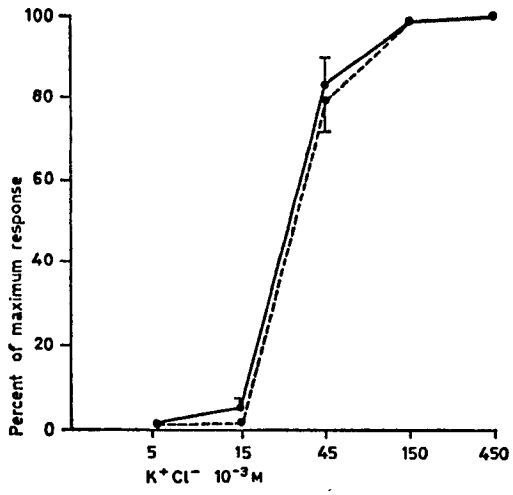

Fig. 3. Dose-response curves for potassium chloride determined in the absence (n = 7) and in the presence (n = 5) of lysergic acid diethylamide (LSD) 6.3×10^{-9} M (------). Vertical bars represent standard errors of the means. Number of experiments are given in parentheses. ——— = Control.

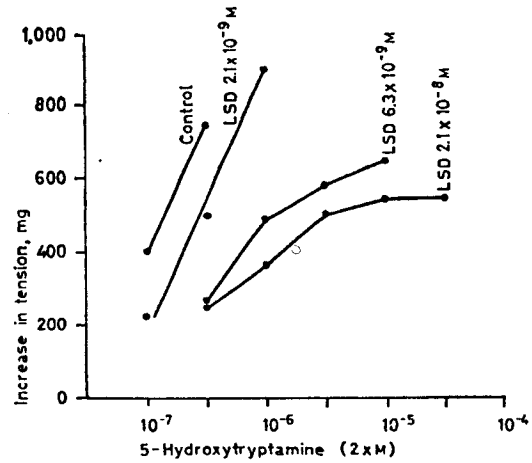

Fig. 4. Dose-response segments for 5-HT determined in the absence and in the presence of progressively increasing concentrations of LSD.

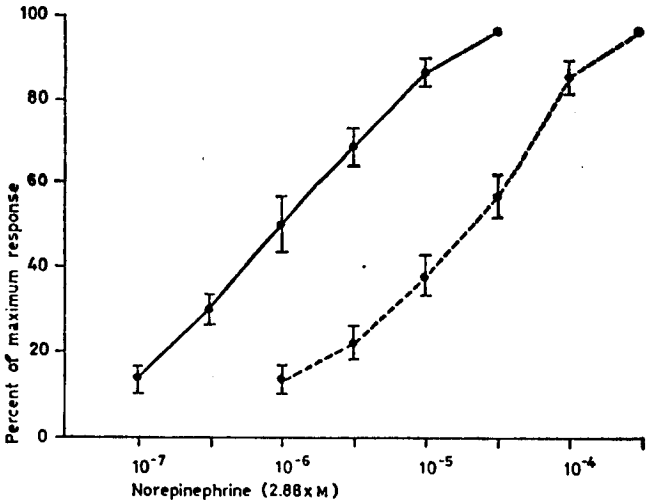

Fig. 5. Dose-response curves for norepinephrine determined in the absence (n = 14) and in the presence (n = 6) of phentolamine 10^{-6} M (------). Vertical bars represent standard errors of the means. Number of experiments are given in parentheses. ——— = Control.

mined at the 500-mg level of response and the results expressed as a plot of log (x − 1) versus the negative log of the molar concentration of antagonist. The regression line obtained from 5 experiments had a slope of 1.56, clearly greater than the theoretical value of 1, expected if the agonist-antagonist interaction were of the equilibrium competitive type. It intercepted the horizontal axis at a point corresponding to 8.7 in the scale of the log molar concentration of antagonist.

Effect of Phentolamine on the Response to Norepinephrine

Phentolamine (10^{-6} M) displaced the control curve for NE 17.7-fold to the right (fig. 5). The geometric mean ED_{50} (95% confidence interval) for NE in the absence and in the presence of phentolamine were 3.5×10^{-6} M (1.6–7.7) and 6.3×10^{-5} M (3.0–12.9), respectively. The maximal tensions developed in the absence and the presence of antagonist were 345 ± 32 mg (n = 14) and 580 ± 88 mg (n = 6), respectively; the difference in maximal tensions was significant ($p < 0.025$). From the dose ratio of 17.7 observed in the presence of phentolamine (10^{-6} M) an apparent Kb value of 5.98×10^{-8} was obtained. The negative logarithm of this Kb value is 7.23.

In this determination, β-adrenergic receptors were not blocked since activation of those receptors failed to elicit relaxation. To the middle cerebral

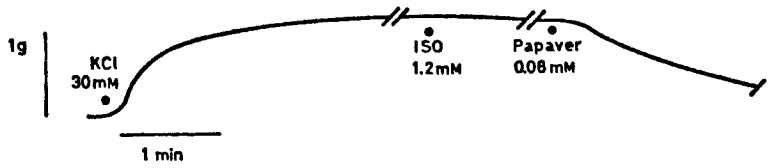

Fig. 6. Tension development of the isolated middle cerebral artery of the goat induced by potassium chloride 30 mM. Isoproterenol (ISO) 1.2 mM and papaverine (Papaver) 0.08 mM were added as indicated when developed tension had plateaued.

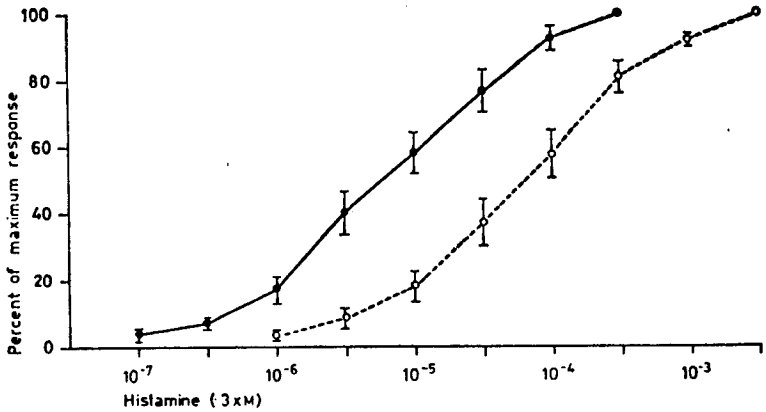

Fig. 7. Dose-response curves for histamine obtained in the absence (n = 14) and in the presence (n = 6) of diphenhydramine 3.4×10^{-7} M (○). Vertical bars represent standard errors of the means. Number of experiments are given in parentheses. ● = Control.

artery of the goat partially contracted by KCl (30 mM), isoproterenol (1.2 mM) did not cause relaxation even though papaverine (0.08 mM) quite clearly did so in a series of 5 experiments (see Discussion; fig.6).

Effect of Diphenhydramine on Response to Histamine

The dose response curve for histamine determined in the presence of diphenhydramine (3.4×10^{-7} M) was shifted 9.6-fold to the right of the control curve (fig. 7). In the absence of antagonist, the geometric mean ED_{50} (95% confidence interval) for histamine was 1.12×10^{-5} M (0.025 – 5.10), and the corresponding value in the presence of diphenhydramine was 1.10×10^{-4} M (0.39–3.0). Tension developments induced by histamine in the absence and in the presence of antagonist were 2,370 ± 389 mg (n = 4) and 1,936 ± 33 mg (n = 6); the difference was not significant ($p > 0.20$).

The apparent Kb value calculated from the dose ratio of 9.6 induced by diphenhydramine 3.4×10^{-7} M was 3.95×10^{-8}; the negative logarithm of this Kb value is 7.41.

Effects of Angiotensin Amide and Lysine-Vasopressin

Angiotensin II was tested in seven experiments in doses ranging from 2.9×10^{-8} to 9.7×10^{-4} M. No responses were observed even though the tissues responded to 5-HT.

Vasopressin was added in increasing doses from 0.2 to 2 IU/ml. The dose of 0.66 IU/ml produced highly variable response which ranged from 80 to 920 mg in four experiments. Attempts to either construct a dose response relationship or repeat the responses to 0.66 IU/ml consistently failed.

Discussion

Relative Potencies of Biogenic Amines

Of the biogenic amines tested on the middle cerebral artery of the goat, 5-hydroxytryptamine was the most potent agent. The maximum tension developed was of the same order as that to potassium chloride and greater than that to norepinephrine. The maximum contractile response to histamine surpassed that to 5-hydroxytryptamine, but its ED_{50} was approximately 500 times greater than that of 5-hydroxytryptamine.

The order of agonists potencies found in the present work for 5-HT, NE and K corresponds to that determined in the dog cerebral arteries by TODA and FUJITA [22]. Also, the relative potencies of 5-HT, NE and H in the middle cerebral artery of the goat are similar to those reported for the same artery of the cat [18]; histamine, however, induced a greater relative tension development in the cerebral arteries of the goat than in the cat. At least two consistent facts seem to emerge from comparative analyses of the responsiveness of the cerebral arteries of several animal species to vasoactive agents, namely the potency of 5-HT and the small size of the maximal responses attained with norepinephrine.

5-HT – LSD Interaction

The results described above indicate that LSD antagonizes the effects of 5-HT on the cerebral arteries of the goat in a manner that progresses from surmountable to unsurmountable blockade as its concentration is increased. The unsurmountable blockade, however, is reversed by removal of LSD from

the medium. Similar results have been previously reported for the perfused rabbit ear [8] and for the rat uterus [9].

The unsurmountability of the blockade could conceivably be attributed to a nonspecific depression of the contractile mechanism of the vascular smooth muscle cells. This possibility is unlikely in view of the lack of effect of LSD (6.3×10^{-9} M) upon the responses elicited by potassium chloride, which is generally considered to act through depolarization of the cell membrane [3], independently of the common pharmacological receptors [5]. Therefore, the observed effects of LSD upon the responses to 5-HT are most likely due to an agonist-antagonist interaction taking place at a step prior to or independent of membrane depolarization. In the sequence of events leading from receptor activation to muscle contraction, the step affected by LSD is specific to activation of the tryptamine receptors. Furthermore, the antagonistic action of LSD appears to be specific for the tryptamine receptors, since in the presence of marked unsurmountable antagonism to 5-HT in vascular and uterine muscle, the effects of epinephrine on oxytocin, respectively, were unchanged [8].

The progression from surmountable to unsurmountable blockade produced by graded doses of LSD is reminiscent of the antagonism exerted by increasing doses of β-haloalkylamines against histamine in the guinea pig ileum [17] and against epinephrine in the rabbit aortic strips [6]. However, the effects of the β-haloalkylamines cannot be reversed by repeated washing. The attachment of LSD to the receptors is reversible, but the blockade produced by LSD (6.3×10^{-9} and 2.1×10^{-8} M) cannot be overcome even by high doses of 5-HT. This kinetic behavior suggests that 5-HT and LSD are most likely interacting with different sites of the receptor molecules or complex, possibly, in an analogous manner to the allosteric interactions between substrate and inhibitor on enzyme systems [16]. Within this context, the fact that the blockade produced by low doses of LSD is overcome by 5-HT could be explained in terms of availability of a given fraction of the receptor population unaffected by the antagonist.

Norepinephrine-Phentolamine Interaction

It is generally accepted that in order to insure the precision of the measurement of the Kb of the phentolamine-receptor complex, precautions should be taken to avoid either concomitant activation of β-adrenergic receptors or loss of the agonist from the biophase due to neuronal uptake [7]. In the present study, such precautions were not taken for the following reasons: (1) Isoproterenol, a β-adrenergic stimulant, failed to elicit relaxation of the

middle cerebral artery of the goat partially contracted by a moderate concentration of KCl (30 mM). Furthermore, isoproterenol added in wide concentration range (10^{-7} to 10^{-3} M) to vascular segments not exposed to KCl, also failed to induce changes in tension [MARCO and URQUILLA, unpubl. observations]. (2) In some vascular smooth muscle at least, neuronal uptake of exogenously added NE exerts only limited influence on the concentration of this amine at the receptor [26]. Apparently this is due to the confinement of the adrenergic nerve endings to the adventital border of the media layer. A similar spatial relationship between nerve terminals and effector cells has been revealed by histochemical fluorescence studies carried out in the cerebral blood vessels of the rabbit [19]. Furthermore, pA_2 values for Ph in rabbit aortic strips in which NE was the agonist were unchanged by inhibition of neuronal uptake with cocaine [24].

The tension development produced by NE was the smallest of all the maximal responses obtained with biogenic amines or potassium ions. The low potency of NE and the limited magnitude of its maximal effects are features consistently observed in the cerebral blood vessels of various animal species [14, 18, 20, 22].

Phentolamine (10^{-6} M) produced a 17.7-fold shift to the right of the control dose-response curve for NE, presumably due to an equilibrium competitive agonist-antagonist interaction. The negative log of the apparent Kb calculated from the dose ratio at the ED_{50} level is 7.23. This figure is relatively close to the pA_2 values of 7.25 and 7.15 obtained with phentolamine in control and denervated cat nictitating membrane, respectively [10]. The pA_2 value for phenylephrine-phentolamine interaction determined by FURCHGOTT [7] in the rabbit aortic strips after 60 min of incubation was 7.8, a value greater than the 7.23 obtained in the present study. This difference is not readily explained, although the time of incubation with the blocking agent might well be a factor influencing the potency of the antagonist. Also, it is important to note that the Kb value determined in the present study was derived from the ratio of ED_{50} for NE, even though the maximal response to NE in the presence of phentolamine was greater than that obtained in the absence of antagonist. This procedure was followed in an attempt to normalize the data and, thus, compensate for variations in the responsiveness of the tissues, but its use affects the precision of the Kb measurement in proportion to the magnitude of the difference in maximal responses.

It appears that the properties of the adrenergic receptors of the cerebral arteries of the goat determining the affinity for a common antagonist are rather similar to those of the corresponding receptors present in the cat nic-

titating membrane. Hence, it seems unlikely that a reduced affinity of NE for the adrenergic receptors of cerebral blood vessels is an important factor contributing to the low activity of NE in this particular vascular bed. Furthermore, the low magnitude of the maximal responses attained with NE can not be attributed to structural factors, such as the relative proportion of muscle to elastic fibers in the cerebral arteries, since considerably higher responses were obtained with histamine, 5-HT and potassium. The mechanism (s) responsible for the low activity of NE in the cerebral vasculature of several animal species remains to be elucidated.

Histamine-Diphenhydramine Interaction

The contraction to histamine was antagonized by diphenhydramine in an equilibrium competitive manner. The negative log of the apparent Kb is 7.41, a value relatively close to the pA_2 value of 7.8 determined with the same antagonist in the guinea pig perfused lung and isolated trachea [1]. The nearly equal potency of diphenhydramine against histamine in these tissues would tend to place their receptors for histamine in the same pharmacological class [1].

Vasoactive Peptides

Angiotensin II was inactive in the isolated middle cerebral artery of the goat. In *in vivo* experiments, intraarterial administration of angiotensin also failed to modify cerebral blood flow, but when it returned to the heart and reached the peripheral vascular system a hypertensive response was observed [LLUCH *et al.*, unpubl. observation]. These results are at variance with those reported by UCHIDA *et al.* [23] in which angiotensin caused constriction of perfused rabbit cerebral arteries followed by 'strong tachyphylaxis'. It is possible that species differences might exist with regard to the reactivity of cerebral vessels to angiotensin; it is also likely that the phenomenon of tachyphylaxis might have been masking the responses, if any, of the cerebral arteries of the goat to this vasoactive peptide.

Lysine-vasopressin produced highly variable responses in the cerebral arteries of the goat followed by tachyphylaxis. Practically the same pattern of activity of vasopressin was found in the rabbit cerebral arteries [23], except that the doses required to elicit responses in the goat were at least 100 times higher than in the rabbit.

In summary, among the biogenic amines tested for their ability to induce contractile effects on the isolated middle cerebral artery of the goat, 5-hydroxytryptamine was the most active and norepinephrine was the least effective.

This vascular preparation seems to possess pharmacological receptors for 5-hydroxytryptamine, norepinephrine and histamine, whose properties are similar to those of the corresponding receptors present in different tissues from various animal species. The receptors for the vasoactive peptides, angiotensin and vasopressin, if present in the cerebral arteries of the goat, were difficult to explore due to tachyphylaxis.

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