J. Pharm. Pharmacol. 1989, 41: 134–135
Communicated March 21, 1988

Distribution of dissociation constant values of muscarinic agonists

ADELE LUCCHELLI, CINZIA BOSELLI, ENZO GRANA, *Institute of Pharmacology, Faculty of Pharmacy, Viale Taramelli 14, 27100 Pavia, Italy*

Abstract—The frequency distributions of dissociation constant values of some muscarinic agonists (carbachol, muscarone and *cis*-2-methyl-5-trimethylammoniummethyl-1,3-oxathiolane) obtained on guinea-pig ileum and atria and rat urinary bladder have been examined to see if the means of the dissociation constant values and the statistical tests for their significance, should be based on geometric rather than on arithmetic means. For the three compounds the distributions on a logarithmic scale did not significantly deviate from normality while the distributions on an arithmetic scale tended to deviate from normality.

In pharmacologic analysis, statistics used to test the significance of differences between samples or treatments are often based on the assumption that the values to be compared are based on normal distributions. The validity of parametric tests such as Student's *t*-test and the analysis of variance depends on this assumption. Fleming et al (1972) have studied the distribution of concentrations that produce 50% of the maximal effect (EC50) of agonists in various tissues and animal species.

They demonstrated that for noradrenaline and acetylcholine the distributions of EC50 values on an arithmetic scale tended to deviate from normality while those on a logarithmic scale did not. Therefore comparisons of agonist potencies with parametric tests must be performed using mean logs (or their antilogs, i.e. the geometric means) and not arithmetic means.

The aim of this work was to see whether the same applied to a pharmacodynamic parameter such as the dissociation constant (K_A) which is widely employed in drug-receptor classifications. Therefore the frequency distributions of K_A values of some muscarinic agonists (carbachol, muscarone and *cis*-2-methyl-5-trimethylammoniummethyl-1,3-oxathiolane (oxathiolane)) obtained on guinea-pig ileum and atria and rat urinary bladder have been studied.

Materials and methods

Male and female guinea-pigs from a local strain (500–600 g) and Wistar-Morini rats (160–220 g) were used.

Guinea-pig ileum and atria and rat urinary bladder were set up as described previously (Grana et al 1986).

Atrial force was recorded isometrically with a Statham force transducer connected to a Battaglia-Rangoni polygraph. The resting tension was adjusted to 1 g. Contractions of guinea-pig ileum and rat urinary bladder were recorded isotonically on an LNI recorder through a Basile transducer. The load applied was 0·5 g for the ileum and 1 g for the bladder.

After the tissues were left to equilibrate cumulative concentration-response curves to an agonist were obtained before and after inactivation of a fraction of receptors with dibenamine 10 μmol L^{-1} (guinea-pig ileum and rat urinary bladder) or 30 μmol L^{-1} (guinea-pig atria), added for times of incubation chosen to reach a reduction of the maximum response to the agonist by 40–80% of the predibenamine control. Only one agonist was tested on each tissue before and after treatment with dibenamine.

K_A values were determined according to the method of Furchgott & Bursztyn (1967) using a computerized graphic method (Zaborowsky et al 1980) with the experimental points chosen according to Thron (1970). Further details on methodology are found in an earlier publication from our laboratory (Grana et al 1987). The data already published together with some others obtained with the same procedure have been the object of the statistical evaluation here reported.

Arithmetic means and 95% confidence limits were calculated for each group of experiments. K_A values were converted to logs. The means and 95% confidence limits were calculated and converted to their antilogs to yield geometric means. The Kolmogorov-Smirnov test was used to evaluate the deviation from normal theoretical distribution of the sample distributions.

The test is based on cumulative frequency distributions of observed and theoretical curves and on the calculation of a D value which indicates the point at which the theoretical and observed curves show the greatest difference (Siegel 1956). P less than 0·05 was chosen as the significance criterion.

Results

Arithmetic and geometric means of K_A values and their 95% confidence limits for carbachol, muscarone and oxathiolane in the guinea-pig atria and ileum and in the rat urinary bladder are presented in Table 1 together with the results of the Kolmogorov–Smirnov test.

For each of the groups of data there is a difference between arithmetic and geometric means, the former being greater than the latter in each instance.

As far as the arithmetic distribution is concerned significant deviations from normality were found for muscarone in guinea-pig atria and ileum and oxathiolane in guinea-pig ileum ($P < 0·05$). For the remainder groups no significant deviation from normality was found ($P > 0·20$).

In none of the groups the distribution of log K_A values presented significant deviation from normality ($P > 0·20$ in each instance).

An example of the difference found in the arithmetic and logarithmic distributions of K_A values is presented in Fig. 1, for oxathiolane in guinea-pig ileum.

Discussion

The data presented have shown that for the three cholinergic compounds tested in three different tissues the distributions of log values of their dissociation constants do not significantly deviate from normality.

On the contrary, the distributions of the arithmetic values deviated significantly from normality in some cases while in others they were normal.

Moreover, in eight out of nine cases the Kolmogorov–Smirnov D value evaluated for the arithmetic distributions is greater than the D value of the corresponding logarithmic distributions which corresponds to a $P = 0·02$ for a one-tailed binomial probability.

These data extend those previously reported by Fleming et al (1972) on the distribution of EC50 values of agonists which resulted always normally distributed only on a logarithmic scale. Thus, comparisons of potency values or of dissociation constant values of different drugs or their variations to the same drug after various treatments based on parametric statistic tests are likely not to be accurate and dependable when done on arithmetic

Correspondence to: A. Lucchelli, Institute of Pharmacology, Faculty of Pharmacy, Viale Taramelli 14, 27100 Pavia, Italy.

Table 1. Distribution of dissociation constant (K_A) values on arithmetic and logarithmic scales.

			Arithmetic			Logarithmic		
Tissue	Agonist	N*	Mean K_A (M) [a](95% C.L.)	D**	P	Geometric mean K_A (M) [a](95% C.L.)	D**	P
Guinea-pig atria	Carbachol	14	$1{\cdot}25 \times 10^{-6}$ (0·81–1·61)	0·310	=0·10	$1{\cdot}05 \times 10^{-6}$ (0·74–1·48)	0·140	>0·20
	Muscarone	15	$2{\cdot}53 \times 10^{-7}$ (1·02–4·04)	0·350	<0·05	$2{\cdot}04 \times 10^{-7}$ (1·07–3·89)	0·140	>0·20
	Oxathiolane	15	$1{\cdot}97 \times 10^{-6}$ (1·31–2·63)	0·120	>0·20	$1{\cdot}51 \times 10^{-6}$ (0·91–2·51)	0·130	>0·20
Guinea-pig ileum	Carbachol	24	$4{\cdot}46 \times 10^{-6}$ (2·91–6·01)	0·180	>0·20	$3{\cdot}02 \times 10^{-6}$ (1·95–4·68)	0·090	>0·20
	Muscarone	29	$7{\cdot}62 \times 10^{-7}$ (4·09–11·15)	0·300	<0·05	$4{\cdot}47 \times 10^{-7}$ (2·95–6·76)	0·140	>0·20
	Oxathiolane	18	$6{\cdot}33 \times 10^{-7}$ (3·76–8·90)	0·420	<0·05	$4{\cdot}47 \times 10^{-7}$ (2·75–7·24)	0·130	>0·20
Rat urinary bladder	Carbachol	34	$2{\cdot}66 \times 10^{-5}$ (1·95–3·37)	0·210	>0·10	$2{\cdot}14 \times 10^{-5}$ (1·70–2·69)	0·09	>0·20
	Muscarone	11	$6{\cdot}05 \times 10^{-7}$ (4·80–7·30)	0·220	>0·20	$5{\cdot}78 \times 10^{-7}$ (4·68–7·08)	0·180	>0·20
	Oxathiolane	14	$5{\cdot}02 \times 10^{-6}$ (2·70–7·34)	0·180	>0·20	$3{\cdot}72 \times 10^{-6}$ (2·29–6·03)	0·140	>0·20

[a] 95% confidence limits.
*Number of observations.
** D value from Kolmogorov–Smirnov test.

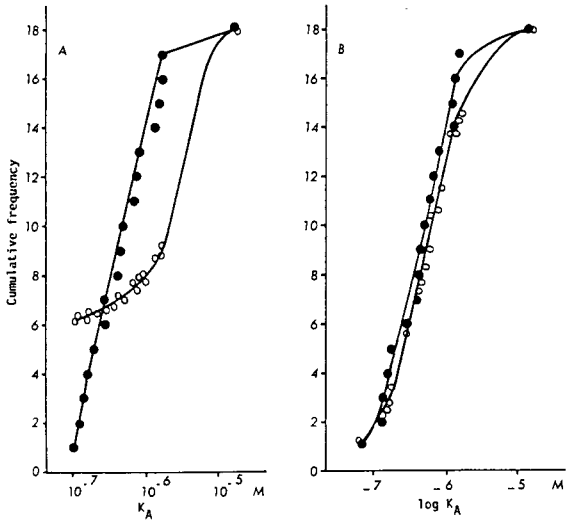

FIG. 1. Cumulative frequency distribution of dissociation constants (K_A) (A) and of the corresponding log values (B) of oxathiolane in guinea-pig ileum. (●) observed scores; (○) expected scores. Arithmetic distribution deviates from normality while log distribution does not.

means. It is therefore necessary when dealing with dissociation constant values to use geometric means or arithmetic means of logarithmic values which are the statistically valid ones.

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