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Appendix

The effects of different recording conditions on the estimates of affinity constants of antagonists for acetylcholine receptors in the guinea-pig ileum

ALISON A. BUTT

Summary

The mean of the estimates of log K for one compound recorded isometrically was not significantly different from that of estimates recorded isotonically but with the other compound it was appreciably higher. With both compounds the estimates of log K were bigger when they were based on comparisons in which the agonist had acted for a longer period.

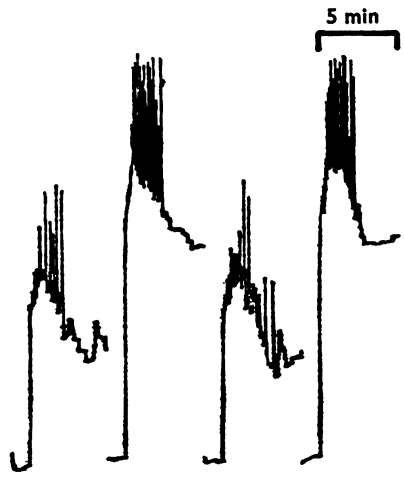


FIG. 1. Isolated guinea-pig ileum recorded isometrically. Responses are shown to 2 and 4×10^{-7}M carbachol. The time intervals were the same as with the bronchial strip preparation (main text, Fig. 1) and the record was interrupted for 7·5 min. during wash and recovery.

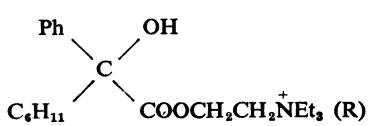
Estimates of the affinity constants of competitive antagonists depend upon measuring the dose-ratio produced by a particular concentration, that is, the ratio of the concentration of agonist producing a response in the presence of the antagonist to the concentration producing the same response when it is absent. Because the response is the same in both situations it might be expected that it should not matter how it is measured, e.g. isotonically or isometrically. The length of time for which the agonist is allowed to act, however, could affect measurements with an antagonist which dissociates slowly from receptors. If there is insufficient time for equilibrium to be established between the agonist and antagonist, the antagonist will, in effect, be acting noncompetitively and produce a bigger dose-ratio and higher estimate of the affinity constant than when the situation is truly competitive. With many antagonists tested on the ileum, especially those with high affinity, it is clear that the 30 s period for which the agonist is in contact with the tissue is too short for equilibrium between agonist and antagonist to be reached. It might, therefore, be expected that measurements with the bronchial strip preparation, with the agonist in contact with the tissue for 5 minutes, would give lower (and more correct) estimates of affinity constants.

Experiments were therefore done in which the ileum was tested under the same conditions as the bronchial strip. It was set up in Krebs solution containing hexamethonium, $2{\cdot}76\times10^{4-}$M, the tension was recorded isometrically, and the agonist, carbachol, allowed to act for 5 minutes. Suitable responses were obtained with 2×10^{-7} and 4×10^{-7}M carbachol, compared with 1×10^{-7} and 4×10^{-7}M on the bronchial strip, and the record (Fig. 1) shows bursts of tension superimposed on the overall increase, but the tension is not sustained and declines considerably with time. In spite of the complexity of the response, it might be expected that the time-course would be the same whether the antagonist is present or not, and the dose-ratios were measured using the responses (but ignoring superimposed

TABLE 7. *Mean values of log affinity constant (±standard error) for acetylcholine receptors in the guinea-pig ileum obtained with the contractions recorded isometrically and by comparing the effects of the agonist (carbachol) after it had been allowed to act for different periods of time*

$(C_6H_{11})_2CHCH_2OCH_2CH_2\overset{+}{N}Et_3$

Time (s)	Isometric	Time (s)	Isotonic
40	7·216±0·095 (9)	30	7·354±0·030 (6) (in Tyrode solution)
80	7·231±0·091 (9)		
300	7·404±0·065 (9)		



C_6H_{11} $COOCH_2CH_2\overset{+}{N}Et_3$ (R)

40	9·791±0·029 (4)	30	9·600±0·068 (8) (in Tyrode solution)
80	9·895±0·080 (4)	30	9·652±0·059 (7) (in Krebs solution)
300	9·979±0·042 (4)		

The preparations were set up in Krebs solution containing hexamethonium, $2{\cdot}76\times10^{-4}$M. Values obtained with the same compounds recorded isotonically and in the presence of hexamethonium are included for comparison (taken from Tables 2 and 3 of the main text).

spikes) after the agonist had acted for 40 s, 80 s, and for 5 min, and the corresponding values of log K are shown in Table 1. The values obtained isotonically, also in the presence of hexamethonium, are included for comparison.

The estimates of log K all increase the longer the agonist has been allowed to act. With di*cyclo*hexylethoxyethyltriethylammonium the results obtained do not differ significantly from those obtained isotonically in Tyrode solution, but with R-phenyl*cyclo*hexylglycolloylethyltriethylammonium the values obtained with isometric recording are appreciably higher than values in either Tyrode solution or Krebs solution with isotonic recording. The difference is comparable with the difference between the values for this compound on the bronchial muscle and on the ileum (main text, Table 5), so it is doubtful whether this can be used as evidence for slight differences in receptor structure. This work also raises doubts about the accuracy of estimates of log K when any potent compound is tested on similar preparations by the cumulative technique described by Van Rossum & Van den Brink (1963).

The increase in the estimates of log K with the length of time the agonist is allowed to act is exactly the opposite of what would be expected from considering the need for time for the agonist and a potent antagonist to come into equilibrium. It appears that in the presence of the antagonist the decline in tension develops faster and the antagonist therefore appears to produce a bigger effect. If the decline in tension is simply due to fatigue it is difficult to see why it should be affected by an antagonist. It is possible, however, that the increased effects of the antagonist are due to a metaphilic effect of the agonist, carbachol (Rang & Ritter, 1969, 1970).

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