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# A NEW APPROACH TO THE MEASUREMENT AND CLASSIFICATION OF FORMS OF SUPERSENSITIVITY OF AUTONOMIC EFFECTOR RESPONSES

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1 It is proposed that sensitizations of autonomic effectors to agonists by drugs or procedures be considered in two main categories: those involving changes in the effective concentration of agonist at receptors (type I) and those involving changes in the responding tissue beyond the initial combination of agonist and receptors (type II). Type I sensitizations are appropriately described by determining the dose-ratio (horizontal shift of the dose-response curve) and type II sensitizations by assessing the change in the magnitude of the response.

2 The inadequacy of the dose-ratio in assessing sensitizations related to an altered physiology of the responding tissue is illustrated by means of hypothetical examples with particular reference to the slopes of dose-response curves and altered maximal responses.

3 An evaluation of the enhancement of responses of rabbit aortic strips to agonists by reserpine indicates that it is a type II sensitization. The shifts of dose-response curves to noradrenaline, isoprenaline, normetanephrine and 5-hydroxytryptamine after reserpine-treatment, were described both by the dose-ratio and by the increment in the magnitude of the response at various contraction amplitudes. The dose-ratio varied unpredictably for each agonist depending on the response level selected for comparison and also varied between agonists. However, the mm increment in response magnitude after reserpine approximated a constant value. Responses to potassium which by horizontal procedures were assessed among the least increased, were found to be enhanced the most when considered as a type II sensitization.

4 It is concluded that both type I and type II procedures should be applied when dealing with an unidentified sensitization and that the data be critically assessed. The appropriate use of these procedures can aid in identifying and clarifying sensitizations, as well as in elucidating the sequence of steps between receptor activation and response in an effector.

## Introduction

The established procedure for assessing sensitization of responses of an autonomic effector to an agonist by a drug or procedure is to determine the horizontal distance between dose-response curves, analogous to the use of the dose-ratio in the study of drug antagonism This procedure was popularized by Trendelenburg (1963) who commented in his review that 'a meaningful measurement of the sensitizing effect of a drug or procedure can be obtained only by the determination of the horizontal shift of the dose-response'.

This view is now so well accepted that the statement can probably be made that investigators of supersensitivity phenomena are so preoccupied with drug concentrations or changes in concentrations as to suffer from 'horizontal bias'. Evidence will be presented here to show that there are forms of sensitization that are most meaningfully expressed by the determination of dose-ratios (horizontal shift) and others that require the determination of changes in the magnitude of the response. Lack of recognition of the need for more than one approach to the measurement of sensitizations may be obscuring the investigation and proper assessment of whole classes of sensitizations.

## Methods

Rabbit aortic strips were prepared as described previously (Kalsner & Nickerson, 1968) and suspended under 2 g of tension in muscle chambers of 15 ml capacity. The bathing medium was Krebs-Henseleit (Krebs) solution (mM): NaCl, 115.3; KCl, 4.6; $CaCl_2$, 2.3; $MgSO_4$, 1.1; $NaHCO_3$, 22.1; $KH_2PO_4$, 1.1; and glucose, 7.8.

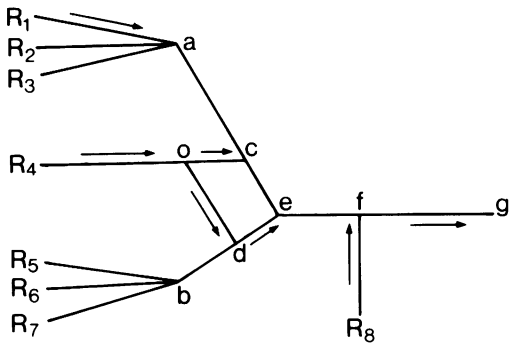


**Fig. 1** A scheme depicting the sequence of steps between agonist receptors (R) and response (g) in an effector.

This was maintained at 37° C and constantly bubbled with a mixture of 95% $O_2$ and 5% $CO_2$. Disodium ethylenediamine tetraacetic acid was added to the Krebs solution to give a final concentration of 10 μg/ml so as to retard heavy metal catalyzed oxidation of catecholamines. The strips were allowed to equilibrate in the muscle chambers for at least 90 min before drug testing. Isotonic contractions were recorded by means of endwriting levers on a slowly moving kymograph drum with a 6.8-fold lever magnification.

Concentrations of (-)-noradrenaline and (−)-adrenaline bitartrates, (±)-normetanephrine and (±)-isoprenaline hydrochloride and 5-hydroxytryptamine creatinine sulphate are expressed in terms of the free base. All drugs are presented as final concentrations in the muscle chambers in g/ml with the exception of potassium chloride which is referred to in terms of molarity. Catechol-*O*-methyltransferase (COMT) was inactivated by incubating strips with U-0521 (3′-4′-dihydroxy-2-methyl propiophenone, Upjohn; 10 μg/ml) and about 10 min later, without washout, agonist testing was resumed. Evidence for the specificity of action of U-0521 as an inhibitor of COMT in aortic strips was provided previously (Kalsner, 1969). Reserpine was dissolved in 10% ascorbic acid, and rabbits were injected intramuscularly with 1 or 2 mg/kg approximately 46 h before they were killed

Dose-response curves were obtained by exposing aortic strips cut to a uniform length (2.5 x 23 mm), to cumulatively increasing concentrations of the agonist under study, without washout of the muscle chambers, until the maximal amplitude of response was reached. Each strip was used for only one dose-response curve and then discarded. Mean values of data are shown with their standard errors. In the absence of an acceptable formal model by which parameters of location (affinity) and scale (intrinsic activity) could be fitted to individual dose-response curves, mean response curves were constructed by averaging the absolute responses (mm) of the different aortic strips. As can be seen in Figs 3 and 4, the reproducibility of the responses from different preparations was such that insignificant distortion of the type suggested for other systems by Ariens, Simonis & Van Rossum (1964), was produced in these mean curves. $ED_{50}$'s were also determined from individual dose-response curves and the geometric means used as representative mean $ED_{50}$'s. The ratio of these values in the control and reserpine-treated groups did not differ materially from those presented in Table 1.

*Theoretical considerations*

*Approaches to the measurement of sensitizations.* A scheme of the pathways from drug receptors to response in a typical effector cell (e.g., smooth muscle of rabbit aorta) is presented in Figure 1. A description of two fairly straightforward types of sensitization occurring at extreme loci in the scheme will illustrate the basis of the present investigation.

*(1) An increase in the effective concentration of agonist at the receptors (R) due to inhibition of a major inactivation pathway.* Such sensitization is most meaningfully expressed by determining the reduction in the administered agonist concentration necessary to produce the same response as in the control condition (e.g., at the 50% response level, $ED_{50}$). This is assessing the horizontal shift of the dose-response curve or dose-ratio. It is obvious that the dose-ratio will vary from agonist to agonist, depending on the relative importance of the inhibited mechanism in reducing its concentration at the receptors. This is illustrated in Figure 2a. All sensitizations due to a change in the drug concentration at receptors (e.g., access barriers, sinks of metabolism, passive binding sites) probably should be assessed horizontally, since this is an appropriate way to determine the degree of alteration in the effective drug concentration. Sensitizations of this sort may be called 'type I sensitizations'.

*(2) A change in the energetics of the crosslinking system of actin and myosin (g) such that a contraction greater than normal develops in response to a given quantity of the standard signal ($Ca^{++}$ available to the filament binding sites).* The crosslinking of actin and myosin is

the final common pathway for the action of all agonists in the model shown in Figure 1. Sensitization due to a change at this level (g) should be observed as a uniform magnification of a given magnitude of contraction, regardless of the agonist or receptor system activated ($R_1$-$R_8$) to achieve the contraction. This is so since the intensity of the signal from f to g, in Fig. 1, must be the same for all contractions of equivalent amplitude elicited through $R_1$-$R_8$. Thus, sensitization should be assessed in the direction of response amplitude. If horizontal measurements are made (shift of dose-response curve along the concentration axis) the sensitization would vary with, among other things, the slopes of the dose-response curves and an erroneous assessment would be made. For example, in Fig. 2b, it is assumed that shortening of the myofibrils is enhanced such that responses to agonists A and B are magnified in amplitude by 100% at any point along the mid-range of response. However, estimating sensitization by determining horizontal shifts at the $ED_{50}$ level would give the erroneous conclusion that responses to A are enhanced by 2.4-fold and those to B by 1.1-fold.

Sensitizations which have as their basis a change in a process beyond receptor excitation probably should be assessed in the direction of response magnitude. Thus, it can be seen that a magnification in the strength of the signal from a to c, or e to f, in Fig. 1, would result in a uniform magnification of a given magnitude of response for all agonists acting on receptor systems $R_1$-$R_3$ or $R_1$-$R_7$, regardless of the shapes of the dose-response curves. Sensitizations of this sort may be called 'type II sensitizations'.

*Limitations of horizontal measurements.* The limitations of horizontal measurements in assessing sensitizations related to changes in the responding cells become obvious, although clearly, they are not restricted to situations where the maximal response to an agonist is altered by the sensitizing procedure. For example, in Fig. 2c, dose response curves to agonists A and B are presented before and after a sensitizing procedure in which the shortening of the myofibrils is enhanced, such that responses are increased in amplitude by 50%, this time uniformly at all levels of contraction, including the maximal. How would the difference between the dose-response curves be assessed by the dose-ratio?

One widely used procedure is to compare the curves at the $ED_{50}$ or other percent response level of each curve (Fleming, 1968; Ozawa & Sugawara, 1970; Altura & Altura, 1971). Generally, this is done by converting responses to percent of maximal response for each curve. Such an analysis

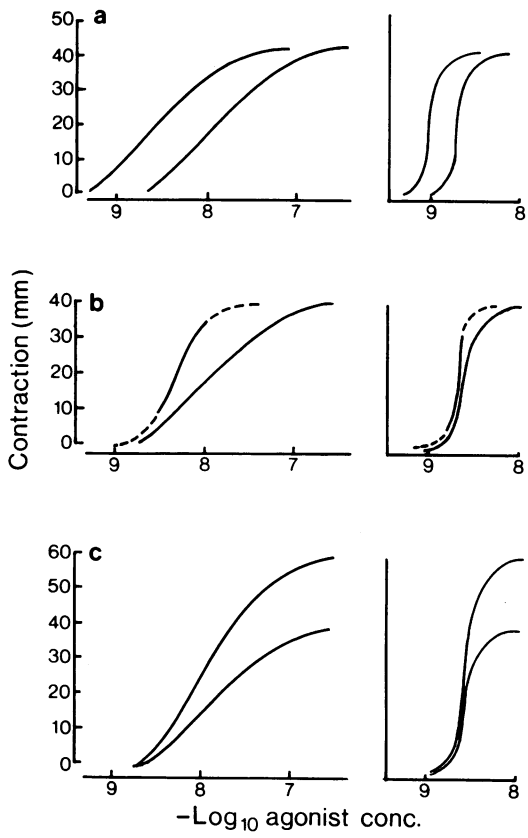


**Fig. 2** Schematic representation of the position of dose-response curves to agonists A (left) and B (right). (a) Before and after a sensitization due to inhibition of an enzyme system which reduces the concentration of these agonists reaching the receptors, at all levels of contraction, by 80% and 50% (dose-ratios of 5 and 2) respectively. (b) Before and after a sensitizing procedure which enhances the energetics of cross-linking of actin and myosin and magnifies contractions along the mid-range of the dose-response curve. Solid portion of dose-response curves after sensitization indicates range of response increased in magnitude by 100% over controls. (c) Before and after a sensitizing procedure which enhances the energetics of cross-linking of actin and myosin and magnifies contractions by 50% along the entire range of response including the maximal.

of the data presented in Fig. 2c would lead to a superimposition of the control and experimental dose-response curves for each agonist. In practice, the distortion of the data produced by such a conversion need not be as obvious as in the example presented here. However, the greater the increase in the maximal response to an agonist, relative to the increase in submaximal responses,

the less will be the sensitization when assessed by horizontal procedures. Further, if the maximal response is increased significantly out of proportion to other points, as is possible (Barnett, Greenhouse & Taber, 1968) conversion of the data to percent of maximal response could lead to the striking and erroneous conclusion that responses at the $ED_{50}$ level are depressed.

Another frequently used procedure is to present the data as mm contraction or g tension and to compare the curves at the $ED_{50}$ or other response level of the control curve and at the response of equivalent amplitude, in mm or g tension on the sensitized preparation (Barnett *et al.*, 1968; Kasuya, Goto, Hashimoto, Watanabe, Munakata & Watanabe, 1969; Haeusler & Haefely, 1970). Such an analysis of the data presented in Fig. 2c would lead to the conclusion that responses to agonist A are enhanced to a greater extent than those to B (e.g., at $ED_{50}$; 1.7 and 1.1-fold), and the increase in maximal response would be unaccounted for. However, if the change in response amplitude is determined at the same level of contraction for both agonists the nature of the sensitization would be apparent.

## Results

### *Analysis of reserpine-induced sensitization*

Reserpine has long been known to sensitize responses of a variety of effectors to certain sympathomimetics and other stimulant drugs. The sensitization is traditionally assessed by horizontal procedures. In the present experiments rabbits were pretreated with reserpine, as described in **Methods**, and dose-response curves to the contractile effects of noradrenaline, normetanephrine and isoprenaline were compared in the control and treated conditions. These three sympathomimetics elicit contractions through activation of the α-adrenoceptors but differ, to some extent, in either the slopes of their dose-response curves or in their maximal amplitudes of response. If the site of action of reserpine is beyond the α-receptors (e.g., beyond $R_1$ in Fig. 1) then for a given intensity of signal elicited at the receptors the amplification of response should be similar for all three agonists. Dose-response curves to the three agonists are presented in Fig. 3 and the data are summarized in Table 1. Comparisons for each agonist made by determining the ratio of $ED_{50}$ control response/ $ED_{50}$ treated response would lead to the conclusion that sensitization is slight and different for each agonist. If sensitization is assessed by reading horizontally across from the $ED_{50}$ or other

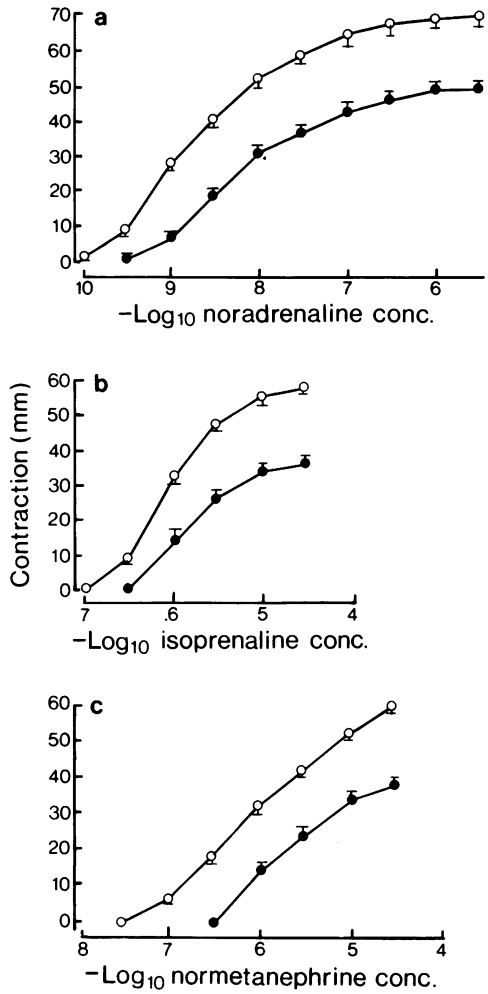


**Fig. 3** Dose-response curves to (a) noradrenaline, (b) isoprenaline and (c) normetanephrine in control (•) and reserpine-pretreated (○) rabbit aortic strips. The number of strips used for each agonist was 13 and 12, 9 and 10 and 10 and 14 in the control and reserpine-pretreated condition, respectively.

level on the control curves to the response of equivalent amplitude in mm on the treated curve different and higher values would be obtained. Comparisons made at some other response level, e.g., $ED_{30}$ or $ED_{70}$, would lead to still other sets of values and, in addition, the ratio of the sensitization for the three agonists would vary at each of the assessed response levels. This is due to the alterations in the slopes of the dose-response curves produced by the reserpine treatment.

However, if sensitization is expressed in the direction of the magnitude of the response

(Table 2) it can be seen that for each agonist, along the mid-range and at the maximal levels of response, the amplification in mm due to reserpine appears to approximate a constant value. Further, the increments in amplitude for the three agonists are comparable.

This study was extended by obtaining dose-response curves to two other agonists, 5-hydroxytryptamine and potassium, acting on other receptor systems. These results are also presented in Fig. 4 and Tables 1 and 2. The increment in the magnitude of the response for 5-hydroxytryptamine is in the same range as that for the α-adrenoceptor stimulants, but for potassium it is considerably greater at the mid-range of response. It is interesting to note that whereas horizontal determinations would give the conclusion that responses to potassium are among the least increased by reserpine, analysis as a type II sensitization reveals that they are increased the most.

*The slope of the dose-response curve and type I sensitizations*

An assumption which is often made in the field of supersensitivity phenomena is that sensitization results in a parallel shift of the dose-response curve (Trendelenburg, 1963). Most workers, therefore, make comparisons at the $ED_{50}$ since this is considered representative of the whole curve. This is probably not the case very often and it could lead to difficulty when determining the dose-ratio for a type I sensitization. As was discussed above the evaluation of a type II sensitization is independent of the shape of the dose-response curve.

Most type I sensitizations probably involve modifications to inactivation pathways and the role of a given pathway in the metabolism of an agonist is unlikely to remain constant over the broad concentration range generally required for a dose-response curve (as much as 10,000-fold). For example, it has been shown that there are three

**Table 1** Analysis of reserpine-induced sensitization

| | *Dose-ratio* | | | | | |
|---|---|---|---|---|---|---|
| | *At equivalent % of maximal response (control/reserpine)* | | | *At equivalent response amplitude in mm (control/reserpine)* | | |
| *Agonist* | $\frac{ED_{30}C}{ED_{30}R}$ | $\frac{ED_{50}C}{ED_{50}R}$ | $\frac{ED_{70}C}{ED_{70}R}$ | $\frac{ED_{30}C}{Equiv.\ mm\ R}$ | $\frac{ED_{50}C}{Equiv.\ mm\ R}$ | $\frac{ED_{70}C}{Equiv.\ mm\ R}$ |
| Noradrenaline (13,12) | 3.0 | 2.9 | 2.7 | 4.5 | 6.3 | 9.5 |
| Isoprenaline (9,10) | 1.6 | 1.6 | 1.5 | 2.3 | 2.9 | 4.0 |
| Normetanephrine (10,14) | 2.5 | 2.1 | 1.4 | 4.8 | 5.3 | 7.0 |
| 5-Hydroxytryptamine (18,12) | 2.7 | 2.7 | 2.4 | 4.5 | 5.0 | 6.9 |
| Potassium (17,12) | 1.7 | 1.6 | 1.5 | 2.1 | 2.0 | 2.0 |

The number of control (C) and reserpine-pretreated (R) strips for each agonist is shown in parentheses.

**Table 2** Analysis of reserpine-induced sensitization

| *Control contraction amplitude (mm)* | *Increment in amplitude due to reserpine (mm)* | | | | |
|---|---|---|---|---|---|
| | *NA* | *ISO* | *NMET* | *5-HT* | *K⁺* |
| 10 | 20.5 | 15.0 | 17.5 | 18.0 | 26.0 |
| 15 | 20.5 | 18.0 | 18.0 | 18.0 | 30.0 |
| 20 | 21.0 | 19.0 | 18.0 | 17.5 | 30.0 |
| 25 | 20.5 | 20.5 | 18.5 | 17.0** | 30.0 |
| 30 | 20.0 | 21.0 | 18.5 | 17.0** | 28.5 |
| 35 | 20.5 | 21.5 | 20.0 | – | 26.0 |
| 40 | 21.0 | – | 21.5* | – | 24.0 |
| 45 | 20.5 | – | – | – | 20.5 |
| 48.3 | 20.0 | – | – | – | – |

* Maximal response to normetanephrine (NMET) in control preparations was 38 mm.
** Maximal response to 5-hydroxytryptamine (5-HT) in control preparations was 27 mm.
The number of control and reserpine-pretreated strips used for each agonist is indicated in Table 1.

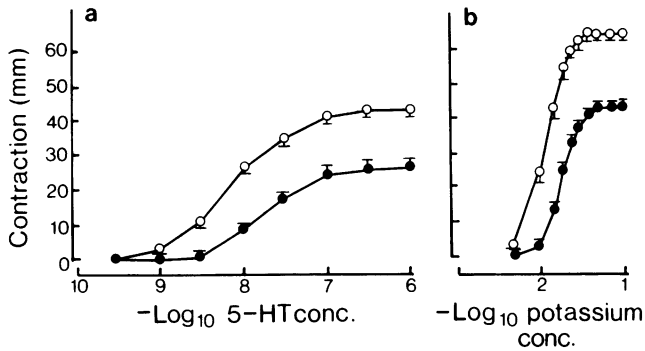


**Fig. 4** Dose-response curves to (a) 5-hydroxytryptamine (5-HT) and (b) potassium in control (•) and reserpine-pretreated (○) rabbit aortic strips. The number of control and reserpine-pretreated strips for each agonist was 18 and 12 and 17 and 12.

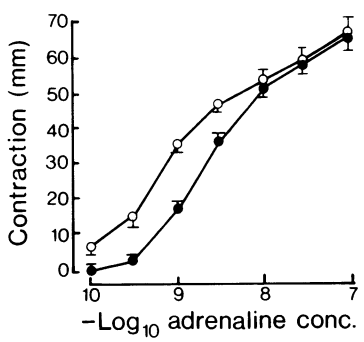


**Fig. 5** Sensitization of responses to adrenaline in rabbit aortic strips after inhibition of catechol-*O*-methyltransferase with U-0521. Responses shown are of 11 control strips (•) and 11 taken from the same aortae and exposed to U-0521 (10 μg/ml) (○).

inactivation pathways of consequence for noradrenaline in rabbit aortic strips (deamination, *O*-methylation and uptake into neuronal and extraneuronal sites) and that the relative importance of these mechanisms changes over the range of concentrations of noradrenaline covered by a complete dose-response curve (Kalsner & Nickerson, 1969a). Similarly, the roles of diamine oxidase and imidazole-*N*-methyltransferase in the inactivation of histamine in aortic strips appears to change with agonist concentration (Kalsner, 1970).

The lack of parallelism in the dose-response curves after inhibition of *O*-methylation, a major inactivation pathway for adrenaline, is evident in Figure 5. The dose-ratio varies depending on the response level chosen for comparison, e.g., at response levels of 10, 30, 50 and 60 mm it is 3.4, 2.9, 1.7 and 0.9. It thus appears that a single value is not necessarily adequate to describe a possible type I sensitization and that a number of points along the dose-response curve need to be compared before a satisfactory assessment can be made.

## Discussion

The only method of assessing sensitization now in general use is to determine the horizontal shift of the dose-response curve (the shift along the concentration axis, the dose-ratio). The widespread acceptance of the dose-ratio in the study of supersensitivity, analogous to its use in drug antagonism, is based on the assumption that sensitizations of effectors can be related to altered concentrations of agonists at receptors. This may be due primarily to the dominance of the presynaptic theory of sensitization. This theory, at its peak, proposed that supersensitivity of autonomic effectors to noradrenaline and other sympathomimetic amines after reserpine-treatment, denervation, decentralization or exposure to a number of agents, such as cocaine, is due solely to the block of neuronal uptake and a consequent diversion of agonist to receptors (e.g., Kopin, 1964; Axelrod, 1965). There is a growing recognition now that many forms of sensitization have as their basis, or involve, changes in the physiology of the responding tissue rather than in the effective receptor concentration of agonist. The enhanced maximal response observed in certain sensitizations, the non-specificity of decentralization and reserpine supersensitivities and evidence for postsynaptic actions of cocaine

and reserpine have been significant factors in this respect (Fleming, 1963; Barnett *et al.*, 1968; Kalsner & Nickerson, 1969b,c).

The present work does not deny the validity of the dose-ratio in assessing sensitization, but points out that it is appropriate only to a special class of sensitizations and that other classes are described more meaningfully by determining the change in the magnitude of the response. It is proposed here that sensitizations be considered in two main categories. Type I sensitizations involve a change in the effective concentration of agonist at the receptor and are best described by determining the dose-ratio. The dose-response curve may or may not be shifted in a parallel way and the dose-ratio should be assessed at several response levels. Type II sensitizations involve changes in the responding tissue beyond the initial combination of agonist and receptor and are evaluated appropriately by determining the change in the magnitude of response for a given level of response. It should be emphasized that the question of whether one should apply type I or type II procedures to any given study in sensitization cannot be made *a priori*. Both procedures should be used and the data critically assessed.

It is important to note that the designation 'type II sensitization' does not imply that all responses along a dose-response curve are magnified by a uniform increment, or that the maximal amplitude of response necessarily is increased. For example, assume that a sensitizing agent is given to aortic strips whose action is to release a fixed amount of bound calcium into the myoplasm (perhaps similar to the way in which low doses of caffeine may enhance skeletal muscle contractions; Sandow, 1965). The free myoplasmic concentration of calcium would be elevated by the same fixed amount, regardless of the degree of contraction produced by the action of an agonist. Sensitization would not be detected as a constant percent increase in the response at all levels, but instead might be a gradually decreasing percent, as the increasing concentrations of agonist release more and more calcium into the myoplasm and reduce the effect of the contribution due to the sensitizing agent. Since one cannot anticipate a uniform increase in magnitude all along the dose-response curve, it is essential that comparisons between agonists be made at equivalent response levels.

The use of type II procedures can serve to characterize a given sensitization and could aid eventually in elucidating the sequence of steps beteen receptor activation and response. For example, if a locus of sensitization is between c and e in Fig. 1, then responses to agonists acting at $R_1$-$R_3$ should be enhanced uniformly and those to agonists acting at $R_5$-$R_8$ not at all. Responses elicited through $R_4$ also would be enhanced but to a lesser extent than those through $R_1$-$R_3$ due to the bifurcation of the signal at o. This can be visualized more easily if one thinks, for instance, in terms of calcium pools in vascular smooth muscle which are activated to different extents by agonists. Some agonists (e.g., potassium) appear to utilize extracellular and/or loosely bound calcium for contraction and others (e.g., angiotensin) mobilize calcium from a more firmly bound store at an intracellular site (Hinke, 1965; Hudgins & Weiss, 1968). Recent evidence indicates that certain agonists interact with both calcium pools (Hudgins & Weiss, 1968; Kalsner, Nickerson & Boyd, 1970). Thus, a sensitization which can be related to calcium mobilization can be analyzed then to provide information on the source of activator calcium for contraction by different agonists and to elucidate the sequence of events between receptor excitation and response.

The analysis of reserpine supersensitivity which is presented here indicates that it can be appropriately considered a type II sensitization and evaluated in the direction of response magnitude. The sensitization is such as to provide a steady, uniform increment in amplitude, regardless of contraction level for all agonists except potassium. Responses to low concentrations of potassium were enhanced to a greater extent than those to other agonists, pointing to a particularly close relationship between the mechanism of potassium action and the locus of reserpine sensitization. In contrast, analysis by the traditional methods of assessing dose-ratios would give the conclusion that sensitization varies unpredictably between agonists and any common denominator in the action of reserpine would be most difficult to isolate.

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## References

ALTURA, B.M. & ALTURA, B.T. (1971). Influence of magnesium on drug-induced contractions and ion content in rabbit aorta. *Am. J. Physiol.*, **220**, 938-944.

ARIENS, E.J., SIMONIS, A.M. & VAN ROSSUM, J.M. (1964). Drug-receptor interaction; Interaction of one or more drugs with one receptor system. In: *Molecular*

*Pharmacology* Vol. 1, ed. Ariens, E.J., pp. 119-286. New York: Academic Press.

AXELROD, J. (1965). Metabolism and inactivation of noradrenaline and adrenaline and the effect of drugs. In: *Proc. 2nd Int. Pharmacological Meeting*, Vol. 3 *Pharmacology of Cholinergic and Adrenergic Transmission*, ed. Koelle, G.B., Douglas, W.W. & Carlsson, A., pp. 205-219. Prague: Pergamon Press and Czechoslovak Medical Press.

BARNETT, A., GREENHOUSE, D.D. & TABER, R.I. (1968). A new type of drug enhancement: increased maximum response to cumulative noradrenaline in the isolated rat vas deferens. *Br. J. Pharmac.*, **33**, 171-176.

FLEMING, W.W. (1963). A comparative study of supersensitivity to norepinephrine and acetylcholine produced by denervation, decentralization and reserpine. *J. Pharmac. exp. Ther.*, **141**, 173-179.

FLEMING, W.W. (1968). Nonspecific supersensitivity of the guinea-pig ileum produced by chronic ganglion blockade. *J. Pharmac. exp. Ther.*, **162**, 277-285.

HAEUSLER, G. & HAEFELY, W. (1970). Pre- and postjunctional supersensitivity of the mesenteric artery preparation from normotensive and hypertensive rats. *Naunyn-Schmiedebergs Arch. Pharmak.*, **266**, 18-33.

HINKE, J.A.M. (1965). Calcium requirements for noradrenaline and high potassium ion contraction in arterial smooth muscle. In: *Muscle*, ed. Paul, W.M., Daniel, E.E., Kay, C.M. & Monckton, G., pp. 269-285. New York: Pergamon Press.

HUDGINS, P.M. & WEISS, G.B. (1968). Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J. Pharmac. exp. Ther.*, **159**, 91-97.

KALSNER, S. (1969). Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. *Circulation Res.*, **24**, 383-396.

KALSNER, S. (1970). Potentiation of responses to histamine in aortic strips by steroids. *Can. J. Physiol. Pharmac.*, **48**, 443-449.

KALSNER, S. & NICKERSON, M. (1968). A method for the study of mechanisms of drug disposition in smooth muscle. *Can. J. Physiol. Pharmac.*, **46**, 719-730.

KALSNER, S. & NICKERSON, M. (1969a). Disposition of norepinephrine and epinephrine in vascular tissue, determined by the technique of oil immersion. *J. Pharmac. exp. Ther.*, **165**, 152-165.

KALSNER, S. & NICKERSON, M. (1969b). Effects of reserpine on the disposition of sympathomimetic amines in vascular tissue. *Br. J. Pharmac.*, **35**, 394-405.

KALSNER, S. & NICKERSON, M. (1969c). Mechanism of cocaine potentiation of responses to amines. *Br. J. Pharmac.*, **35**, 428-439.

KALSNER, S., NICKERSON, M. & BOYD, G.N. (1970). Selective blockade of potassium-induced contractions of aortic strips by B-diethylaminoethyl-diphenylpropylacetate (SKF 525A). *J. Pharmac. exp. Ther.*, **174**, 500-508.

KASUYA, Y., GOTO, K., HASHIMOTO, H., WATANABE, H., MUNAKATA, H. & WATANABE, M. (1969). Nonspecific denervation supersensitivity in the rat vas deferens 'in vitro'. *Eur. J. Pharmac.*, 8 177-184.

KOPIN, I.J. (1964). Storage and metabolism of catecholamines: the role of monoamine oxidase. *Pharmac. Rev.*, **16**, 179-191.

OZAWA, H. & SUGAWARA, K. (1970). Sensitivity of the isolated vas deferens of the guinea-pig to norepinephrine and acetylcholine after denervation, decentralization and treatments by various agents. *Eur. J. Pharmac.*, **11**, 56-66.

SANDOW, A. (1965). Excitation-contraction coupling in skeletal muscle. *Pharmac. Rev.*, **17**, 265-320.

TRENDELENBURG, U. (1963). Supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **15**, 225-276.

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