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Autonomic cardiac control. I. Estimation and validation from pharmacological blockades

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Abstract

Pharmacological blockades have been used to estimate the relative contributions of the autonomic branches to cardiac chronotropic control. Systematic biases in these estimates, however, can arise from both methodological and physiological factors. Selective blockades can be interpreted by two inferential models, and a single blockade condition can yield estimates of autonomic control for both autonomic branches. The residual autonomic control of the heart after blockade of a single division provides an index of the functional contribution of the unblocked branch. In contrast, the change in chronotropic state of the heart after blockade of the same division reflects the subtractive loss of that branch and thus provides an index of the normal contribution of the blocked branch. We demonstrate that the systematic biases that can arise in blockade studies introduce distortions of the subtractive and residual estimates that are of equal magnitude but opposite sign. Consequently, the discrepancy between the subtractive- and residual-model estimates provides a measure of bias in blockade studies and permits the derivation of validity indices that can facilitate interpretations of blockade data.

Descriptors: Autonomic nervous system, Heart period, Cholinergic antagonists, Adrenergic antagonists

The relative contributions of the autonomic branches to basal cardiac chronotropic state and reactive change are of considerable interest, from both basic and applied perspectives. A traditional approach to this issue is through the use of selective pharmacological blockades of the sympathetic and parasympathetic innervations of the heart. This approach has yielded valuable insights into autonomic control, is broadly applicable, and continues to be widely applied in both human and animal studies. Recent applications range from basic baroreflex studies (Fisher, 1989; Head & McCarty, 1987) to the autonomic origins of conditioned responses (Iwata & LeDoux, 1988; Randall, Randall, Brown, Yingling, & Raisch, 1992) to the evaluation of noninvasive indices of cardiac control (Rimoldi, Pierini, Ferrari, Cerutti, Pagani, & Malliani, 1990; Weise, Heyenreich, & Runge, 1987). In fact, the blockade approach represents a fundamental standard for defining autonomic control of the heart, because more direct measures of cardiac nerve activity are generally not feasible in human subjects. A recent advance in the analysis of autonomic control was offered by Stemmler, Grossman, Schmid, and Foerster (1991), who developed a comprehensive quantitative structural model, from blockade data, of distinct autonomic components of psychophysiological response (see also Stemmler, 1993).

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As recognized by Stemmler et al. (1991), however, the blockade approach is not without limitation, and interpretations of results are not always straightforward. Estimates of autonomic control derived from blockades can be systematically biased, for example, by interactions among the branches at the level of the organ, by indirect or reflexive alterations in the unblocked branch, by nonselective actions of the blockers, or by incomplete blockades. Because of these and other potential sources of bias, interpretations of blockade studies may be restricted to a qualitative level. In advancing from qualitative to quantitative analysis, the validity of the autonomic estimates becomes crucial. Interpretations of autonomic estimates derived from blockade data, as for any other psychophysiological metric, revolve around issues of statistical reliability and metric validity. Reliability, related to individual differences, measurement error, and quasi-random variance, can be dealt with through standard statistical approaches. Validity issues, however, have been more difficult to address. In the absence of indices of systematic bias introduced by pharmacological blockades, interpretation of autonomic estimates may be questioned. Although the potential biases in blockade studies are generally recognized, their direct measurement is generally not feasible or prohibitive in most applications.

In the present paper, we extend the quantitative modeling of Stemmler et al. (1991) for the autonomic control of the heart. Our approach capitalizes on the fact that a given blockade condition affords an index of both autonomic branches. The residual autonomic control after pharmacological blockade of a given autonomic branch offers an index of the remaining control of

the unblocked branch. The remaining reactivity after sympathetic blockade, for example, provides an index of the residual parasympathetic contribution to that response (e.g., Equation 4c of Stemmler et al., 1991). In contrast, the change in functional state or response of an organ after pharmacological blockade of the same autonomic division also provides a subtractive index of the normal contribution of the blocked branch. The attenuation of a response after sympathetic blockade, for example, provides a subtractive estimate of the normal sympathetic contribution to that response (e.g., Equation 2c of Stemmler et al., 1991). The use of both sympathetic and parasympathetic antagonists thus provides two separate indices of each autonomic branch. Because the two indices of a given branch are based on distinct blockade conditions (i.e., on blockade of the target branch and the opposite branch), they are subject to separate sets of pharmacological bias. In the present paper, we show that these relationships can provide an important validity metric for blockade analyses. We first outline formal methods for the quantitative estimation of autonomic contributions to basal cardiac state and phasic response. We then develop quantitative indices of bias in autonomic estimates and describe validity metrics for autonomic estimates and their applications in the interpretation of autonomic estimates derived from blockade data. This approach offers a substantial improvement in the application and interpretation of blockade studies.

Derivation of Autonomic Estimates and Bias Indices

The basic logic of blockade studies is relatively straightforward. Selective blockade of a given autonomic branch eliminates the functional influence of that branch on the target organ. Remaining autonomic control after blockade reveals the residual influence of the unblocked branch, and the decrement in autonomic control reflects the subtractive effects of the loss of the blocked branch. The basic data of autonomic blockade studies consist of measures of the functional state of the organ in the unblocked condition after selective sympathetic and parasympathetic blockades and ideally after dual blockade as well. Figure 1A illustrates representative heart period levels under these four blockade conditions for human subjects under sitting and standing conditions (Berntson, Cacioppo, & Quigley, 1993).

Viewed from a residual model, the autonomic control after blockade of a single autonomic branch provides an index of the functional contribution of the unblocked division (illustrated by the solid arrows of Figure 1A; s' = sympathetic control, p' = parasympathetic control). From the vantage of a subtractive model, blockade eliminates the influence of the target branch, and the resulting change in functional state of the organ provides an index of the normal contribution of that branch (illustrated by the dashed arrows in Figure 1A; s'' = sympathetic control, p'' = parasympathetic control). Thus, blockade of a given autonomic division provides an index of the contributions of the unblocked branch via the residual model and an estimate of the blocked branch via the subtractive model. The essential distinction between these inferential models is the reference point from which autonomic control is estimated. With sympathetic blockade, for example, the difference between the organ state at baseline and after sympathetic blockade (arrow s'' in Figure 1A) represents the subtractive effect of the loss of sympathetic influences and provides an index of basal sympathetic control in the unblocked state. In contrast, if the reference is the zero point of autonomic control (the intrinsic functional state of the organ, indexed by dual blockade),[1] then selective sympathetic blockade reveals the residual impact of the parasympathetic branch (arrow p' in Figure 1A).

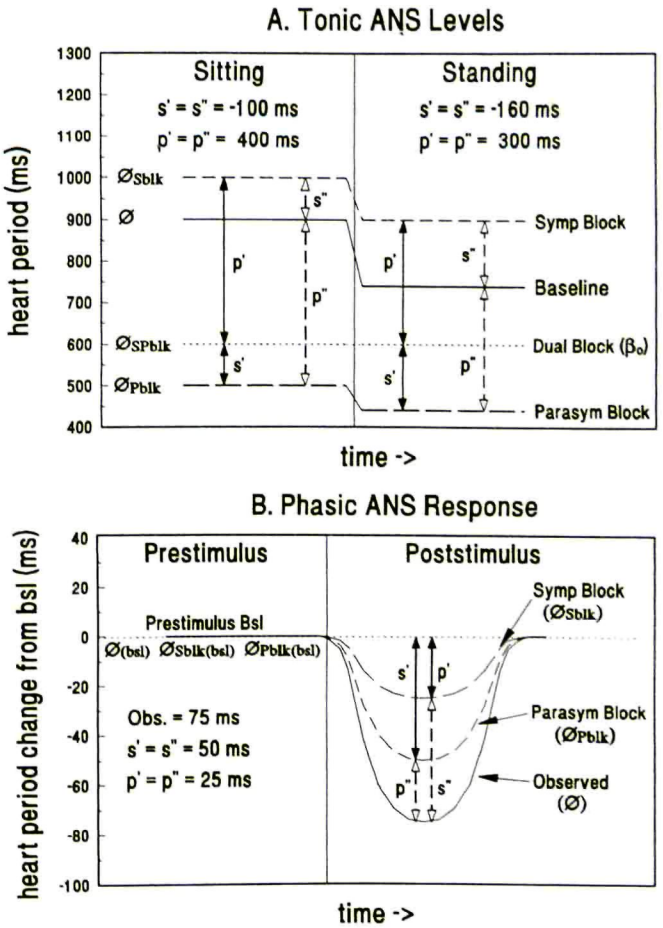

Figure 1. A. Illustrative heart period levels obtained under unblocked conditions, after selective sympathetic and parasympathetic blockades, and after dual blockade. Blockade conditions are listed to the right, and the descriptors used in equations are given on the left. Solid arrows represent residual model estimates of autonomic contributions (s' and p') referenced to the zero point of autonomic control (β_0, indexed by dual blockade). Dashed arrows depict subtractive model estimates (s'' and p'') expressed as a change from unblocked heart period. Heart period levels under sitting and standing approximate values obtained empirically. B. Illustrative phasic heart period response in the unblocked state and after selective sympathetic and parasympathetic blockades. Responses are depicted as changes from the prestimulus levels (bsl) associated with the respective blockade condition rather than in absolute values. Solid arrows represent residual model estimates of autonomic contributions to phasic response (s' and p') referenced to the prestimulus level. Dashed arrows illustrate subtractive model estimates of autonomic contributions to phasic response (s'' and p'') expressed as a difference from the observed unblocked response.

[1] The intrinsic heart period derived from autonomic blockade does not always agree with the results of surgical denervation (Evans, Randall, Funk, & Knapp, 1990). The reasons for these differences are not entirely apparent, although it has been suggested that autonomic nerves may exert some general trophic influence that is not related to the classical receptor actions of the autonomic nerves (Evans, Randall, Funk, & Knapp, 1990).

Formal development. The functional state of the heart at any point in time can be expressed by the following general equation:

$$\phi = \beta + S + P, \tag{1}$$

where ϕ is the chronotropic state (in milliseconds) of heart period, β is the intrinsic heart period in the absence of autonomic control, and S and P are the functional contributions of the sympathetic and parasympathetic divisions, respectively. Equation 1 is not intended to describe the transfer functions relating autonomic outflows to functional effects on the heart (Berger, Saul, & Cohen, 1989; Dexter, Levy, & Rudy, 1989; Levy & Zieske, 1969; Warner & Cox, 1962) nor need it represent a steady state condition (Madwed, Albrecht, Mark, & Cohen, 1989). Rather, the S and P terms reflect the momentary contributions of the autonomic divisions to heart period, including the effect of adrenal catecholamines.

The residual model. Selective autonomic blockades reduce the respective sympathetic or parasympathetic terms (S, P) of Equation 1 to zero and provide estimates of the residual independent influence of the unblocked branch. Assuming complete selective blockades, the following estimates of S and P derive from Equation 1:

$$s' = \phi_{Pblk} - \beta \tag{2a}$$

$$p' = \phi_{Sblk} - \beta, \tag{2b}$$

where ϕ_{Pblk} and ϕ_{Sblk} represent the functional states of the organ under parasympathetic and sympathetic blockade, respectively, and β represents the functional state of the organ in the absence of autonomic control. Dual blockade nulls both the S and P terms in Equation 1 and yields an estimate (β_0) of the intrinsic functional state of the organ (β):

$$\beta_0 = \phi_{SPblk}, \tag{2c}$$

where ϕ_{SPblk} represents the functional state under dual sympathetic and parasympathetic blockade.

Substituting the identity in Equation 2c for the β terms in Equations 2a and 2b yields

$$s' = \phi_{Pblk} - \phi_{SPblk} \tag{3a}$$

$$p' = \phi_{Sblk} - \phi_{SPblk}. \tag{3b}$$

Equations 3a and 3b define the lengths of the s' and p' arrow vectors in Figure 1A and provide a means of estimating sympathetic and parasympathetic control of a target organ.[2] These equations exemplify the residual inferential model, where selective autonomic blockades reveal the operations of the unblocked branch from the reference point of zero autonomic control (β).

The subtractive model. Alternative estimates of autonomic control can be derived by the subtractive approach, where the contribution of the blocked branch is inferred from the difference between the unblocked state and that under selective autonomic blockade. Subtractive estimates of S and P can be derived from Equations 1, 2a, and 2b as the differences in functional state between the unblocked condition and selective blockades:

$$s'' = \phi - \phi_{Sblk} \tag{4a}$$

$$p'' = \phi - \phi_{Pblk}. \tag{4b}$$

These equations define the lengths of the s'' and p'' arrows of Figure 1A from the reference point of the unblocked baseline state.

Potential biases in blockade studies. In the absence of error biases, the residual and subtractive estimates of sympathetic control (Equations 3a and 4a) can be shown to be formally equivalent. The same is true for the residual and subtractive estimates of parasympathetic control (Equations 3b and 4b). Biases in these estimates, however, may be introduced by autonomic blockades. These biases can arise from at least four general sources: a) interactions among the autonomic branches at the level of the target organ, b) indirect blockade-induced alterations of the unblocked division, c) nonselective actions of the pharmacological blockers, and d) incomplete blockades. These classes of bias are not entirely independent mechanistically but represent the general origins of potential bias in blockade studies.

One source of bias in blockade studies lies in the potential interactions among the autonomic branches at the level of the target organ. Mutual inhibitory interactions in the autonomic control of the heart have been demonstrated between the sympathetic and parasympathetic divisions (Hall & Potter, 1990, Levy, 1984; Manabe et al., 1991; Warner & Levy, 1989). Interactions impact on autonomic control when both branches are active but can be eliminated when one or both branches are pharmacologically blocked. Consequently, autonomic estimates derived from blockade studies may not appropriately reflect the true impact of the branches in the unblocked state (see also Stemmler et al., 1991).[3]

[2] The signs here are not arbitrary but relate to the direction of sympathetic and parasympathetic effects on heart period. The sympathetic and parasympathetic estimates must be represented by the appropriate signs (negative and positive, respectively) for the basic identity in Equation 1 to hold ($\phi = \beta + S + P$). For example, given an intrinsic heart period (β) of 600 ms (ϕ_{SPblk}) and a measured heart period under parasympathetic blockade (ϕ_{Pblk}) of 500 ms, Equation 3a yields a residual estimate (s') of −100 ms (that is heart period shortening). If the corresponding residual parasympathetic estimate (Equation 3b) yielded +200 ms, it would be intuitively apparent that the unblocked heart period should be longer than the intrinsic period because parasympathetic control is greater than sympathetic control. This relationship is expressed in Equation 1 (i.e., $\phi = \beta + S + P = 500 + [-100] + 200 = 600$). Using unsigned values, however, would give a derived estimate of 800 ms, which is incorrect. The signs in Equation 1 could be expressed to appropriately reflect the opposing effects of the branches on heart period, that is, $\phi = \beta - S + P$. This expression would be acceptable but would not preclude the need to maintain the sign of the estimates. For phasic (change) estimates, sympathetic control could either increase or decrease, which would yield opposite effects on heart period. Expressing the sympathetic estimate as an absolute value would not distinguish between these opposite effects and would hopelessly corrupt the estimates. The formal derivations are correct only if the signs are retained. The same arguments apply to estimates of error bias.

[3] Interactions would bias autonomic estimates only to the extent to which they are mediated by the same receptor types that underlie the direct postsynaptic response and hence are susceptible to the same blockades. This may not always be the case, as with the apparent neuropeptide Y-mediated sympathetic inhibition of parasympathetic control (Hall & Potter; 1990, Warner & Levy, 1989). To the extent to which interactions survive blockades, they introduce no direct biases in autonomic estimates.

A second class of bias arises if blockade of one autonomic division yields reflexive adjustments in or otherwise alters activity of the unblocked branch. Sympathetic blockade, for example, may alter blood pressure and yield a baroreflex alteration of vagal activity. Such reflex adjustments may systematically bias the parasympathetic estimate, as derived from the sympathetic blockade condition. Additional biases in blocker studies can arise from nonselective receptor actions of blockers. Competitive antagonists show various degrees of receptor specificity, and nonselective actions typically increase at higher doses. Nonselective actions can also arise from the fact that receptor classes mediating autonomic actions on the heart are also present in tissues remote from the target. Central actions of autonomic blockers, for example, may alter autonomic control directly by effects on central cardiovascular mechanisms or indirectly by alterations of behavioral state (e.g., induction of anxiety). Central actions can be minimized by careful selection of doses and by the use of quaternary agents, or agents with low lipophilicity, that do not readily penetrate the central nervous system. Diffusion barriers to these agents are at best relative, however, and even quaternary compounds can exert central actions (Moore, Dudchenko, Comer, Bruno, & Sarter, 1992).

Additional biases can arise from incomplete blockades of the autonomic innervations. Incomplete blockades lead to subtractive model underestimates and residual model overestimates of the contributions of the autonomic branches. Although the problem of incomplete blockades can be minimized by thorough Dose × Response studies and careful selection of doses, this issue is nontrivial. Competitive blockades are never absolute, and an effective blockade for a moderate response may not be complete at higher levels of activation. Although the degree of blockade can be increased by higher doses of the antagonist, higher doses also tend to diminish blocker selectivity. Consequently, selection of an appropriate dose may entail a compromise between completeness and selectivity.

Bias terms in autonomic estimates. Although it is advisable to minimize biases whenever feasible, it may not be possible to completely eliminate bias in blockade studies. In the absence of methods for estimating biases, interpretations may be limited to the qualitative level. The residual and subtractive models outlined above offer quantitative estimates of sympathetic and parasympathetic control and, of greater significance, permit the quantitative estimation of systematic bias in blockade studies.

Indices of S and P as derived from the residual model (Equations 3a and 3b) and the subtractive model (Equations 4a and 4b) provide a means of quantifying autonomic control. Random errors, including measurement error,[4] are inherent in any empirical estimates of S and P and can be dealt with by standard experimental (e.g., increasing sample size, control of extraneous variables) or statistical (e.g., reliability estimates or other variance indices) methods. Random errors are assumed in the following equations but do not systematically bias estimates. For clarity of exposition, terms for random error are not included in the equations below. Explicit terms must be added to the estimates, however, to represent systematic biases arising from pharmacological blockades. Specifically, estimates of sympathetic (s') and parasympathetic (p') control from the residual model derivations of Equations 2a and 2b become

$$s' = \phi_{Pblk} - \beta + \epsilon_{Pblk} \tag{5a}$$

$$p' = \phi_{Sblk} - \beta + \epsilon_{Sblk}, \tag{5b}$$

where ϵ_{Pblk} and ϵ_{Sblk} are the error terms that represent the biases associated with parasympathetic and sympathetic blockades.

The error terms ϵ_{Pblk} and ϵ_{Sblk} can include biases from interactions, indirect effects on the unblocked branch, nonselective actions of the autonomic blockers, and incomplete blockade. As in Equations 3a and 3b, we can substitute the β_0 estimate derived from dual blockades (ϕ_{SPblk}, Equation 2c) into the above equations. The estimate β_0 can also be biased, and an error term for this condition also needs to be added to Equations 5a and 5b. The error term for dual blockade (ϵ_{SPblk}), however, includes only biases from incomplete blockades. Because both branches are blocked, interactions do not impact on this estimate nor could reflexive adjustments or other indirect alterations manifest in the functional state of the organ. Similarly, nonselective actions would have no autonomic route to influence functional state.[5] Substituting the β_0 estimate and its error term, the computational residual model estimates for sympathetic and parasympathetic control become:

$$s' = \phi_{Pblk} - \phi_{SPblk} + \epsilon_{Pblk} - \epsilon_{SPblk} \tag{6a}$$

$$p' = \phi_{Sblk} - \phi_{SPblk} + \epsilon_{Sblk} - \epsilon_{SPblk}, \tag{6b}$$

where ϕ_{SPblk} is the dual blockade estimate of β from Equation 2c and ϵ_{SPblk} is the error term for the dual blockade condition.

Error terms are also necessary for the subtractive model estimates of sympathetic (s'') and parasympathetic (p'') control. For the subtractive model derivations, both ϕ_{Pblk} and its error term (ϵ_{Sblk}) would be subtracted from ϕ in Equation 5a, and both ϕ_{Pblk} and its error term (ϵ_{Pblk}) would be subtracted in Equation 5b, yielding

$$s'' = \phi - \phi_{Sblk} - \epsilon_{Sblk} \tag{7a}$$

$$p'' = \phi - \phi_{Pblk} - \epsilon_{Pblk}. \tag{7b}$$

There are several important features inherent in the above equations. As is apparent in Equations 6 and 7, selective blockades of both autonomic divisions provide two separate estimates of each branch, one derived from the residual model (prime estimate) and one from the subtractive model (double prime estimate). In the absence of error bias, the corresponding prime

[4] Systematic measurement errors, as for any other study, could also bias results. The present methods are designed to identify systematic biases associated with blockade conditions and could not be expected to immunize a study against any and all sources of bias. Hence, the application of the present approach does not eliminate the need for rigor of measurement. Systematic measurement errors that are selectively associated with a blockade condition, however, would be appropriately indexed by the methods described here. An example would be nonlinearities in timing systems that disproportionately bias heart period interval measures of faster heart beats, such as those seen after atropine administration.

[5] Some beta adrenergic blockers exert direct actions of the myocardium, and these actions can bias autonomic estimates. More recently developed selective beta$_1$ antagonists, such as atenolol, appear to have minimal direct effects on myocardial membranes (Barrett, 1985; Frishman, 1982). In any event, nonautonomic effects of blockers can be detected as biases, as will be apparent in the subsequent analyses.

(s', p') and double prime (s'', p'') estimates can be shown to be formally equivalent. The prime and double prime estimates of a given branch derive from different blockades, however, and are thus differentially biased by the blockade conditions. This differential bias allows a quantitative specification of the magnitude of confound in blockade studies, which is illustrated by an application of the general approach to the analysis of phasic autonomic response.

Analysis of Phasic Autonomic Response

Equations 6 and 7 (see also Table 1) provide quantitative estimates of the contributions of the autonomic branches to chronotropic state. We first consider the applications of the residual and subtractive models to the analysis of phasic autonomic response and to the derivation of error terms representing systematic biases in the estimates of autonomic control. This analysis requires that the chronotropic response in question be measured under unblocked conditions and after selective sympathetic and parasympathetic blockades. Figure 1B illustrates a simulated heart period response to an experimental stimulus (ϕ, solid line), expressed as a change from prestimulus baseline ($\phi[bsl]$). Also illustrated are the responses observed under selective blockades of the two autonomic branches (ϕ_{Sblk} and ϕ_{Pblk}, dashed lines), again expressed relative to their respective prestimulus levels ($\phi_{Sblk}[bsl]$ and $\phi_{Pblk}[bsl]$). These three time-varying responses comprise the basic data for analysis.

Residual model analysis. The response obtained under selective blockade of a single autonomic division provides an index of the phasic response of the unblocked branch. In Figure 1B, this index is illustrated for the peak response by the arrows s' and p', which depict the residual model estimates of sympathetic and parasympathetic contributions to the response at the response peak. For analysis of phasic response, the absolute level of autonomic control is not of primary concern. Rather, the change in sympathetic control from baseline to the response at time t characterizes the contribution of that branch to the phasic response. The quantitative index of the sympathetic contribution is a change score of the form

$$\Delta s'(t) = s'(t) - s'(bsl),$$

where $\Delta s'(t)$ represents the change in sympathetic control from prestimulus baseline $s'(bsl)$ at time t. From Equation 5a, the value of $\Delta s'(t)$ can thus be expressed as follows:

$$\Delta s'(t) = [\phi_{Pblk}(t) - \beta + \epsilon_{Pblk}(t)] - [\phi_{Pblk}(bsl) - \beta + \epsilon_{Pblk}(bsl)],$$

where $\phi_{Pblk}(bsl)$ is the prestimulus (baseline) chronotropic state under parasympathetic blockade and $\epsilon_{Pblk}(bsl)$ is the error term for the prestimulus state. Simplifying this equation

Table 1. *Symbolic Notations and Computational Formulas*

Symbolic notation	Parameter
S, P	Sympathetic and parasympathetic control, respectively
s', p'	Residual model estimates of S and P, respectively
s'', p''	Subtractive model estimates of S and P, respectively
β (beta)	Intrinsic heart period in the absence of autonomic control
ϕ, $\phi_{(t)}$	Basal heart period and heart period at time t, respectively
ϕ_{Sblk}, ϕ_{Pblk}, ϕ_{SPblk}	Heart period under sympathetic, parasympathetic, and dual blockades, respectively
ϵ_{Sblk}, ϵ_{Pblk}, ϵ_{SPblk}	Bias estimate for sympathetic, parasympathetic, and dual blockades, respectively
$X(bsl)$	Value of X during preevent baseline
$X(t)$	Value of X at time t

	Tonic estimates	
Parameter	Equation	Computational form
Intrinsic heart period (β)		$= \phi_{SPblk}$
Sympathetic Control (S)	$S = (s' + s'')/2$	$= [(\phi_{Pblk} - \phi_{SPblk}) + (\phi - \phi_{Sblk})]/2$
Parasympathetic Control (P)	$P = (p' + p'')/2$	$= [(\phi_{Sblk} - \phi_{SPblk}) + (\phi - \phi_{Pblk})]/2$
Error Bias for S and P	$\epsilon_{blk} = \lvert s' - s'' \rvert/2$	$= \lvert(\phi_{Pblk} - \phi_{SPblk}) - (\phi - \phi_{Sblk})\rvert/2$
	$= \lvert p' - p'' \rvert/2$	$= \lvert(\phi_{Sblk} - \phi_{SPblk}) - (\phi - \phi_{Pblk})\rvert/2$

	Phasic estimates	
Parameter	Formal equation	Computational form
Sympathetic response, $\Delta S(t)$	$\Delta S(t) = [\Delta s'(t) + \Delta s''(t)]/2$	$= [\phi_{Pblk}(t) - \phi_{Pblk}(bsl) + \phi(t) - \phi_{Sblk}(t)]/2$
Parasympathetic response, $\Delta P(t)$	$\Delta P(t) = [\Delta p'(t) + \Delta p''(t)]/2$	$= [\phi_{Sblk}(t) - \phi_{Sblk}(bsl) + \phi(t) - \phi_{Pblk}(t)]/2$
Error bias for $\Delta S_{(t)}$ and $\Delta P_{(t)}$	$\Delta\epsilon_{blk}(t) = \lvert\Delta s'(t) - \Delta s''(t)\rvert/2$	$= \lvert\phi_{Pblk}(t) - \phi_{Pblk}(t) - \phi(t) + \phi_{Sblk}(t)\rvert/2$
	$= \lvert\Delta p'(t) - \Delta p''(t)\rvert/2$	$= \lvert[\phi_{Sblk}(t) - \phi_{Sblk}(t)] - [\phi(t) - \phi_{Pblk}(t)]\rvert/2$

Validity coefficient[a] $v_{\delta} = \dfrac{|effect\ size|}{|effect\ size| + |error\ bias|}$

[a] *Effect size* is the size of the experimental contrast, and *error bias* is the relevant ϵ_{blk} value.

and following the convention $\Delta\epsilon_x = \epsilon_x(t) - \epsilon_x(bsl)$ yields an estimate of the phasic sympathetic contribution:

$$\Delta s'(t) = \phi_{Pblk}(t) - \phi_{Pblk}(bsl) + \Delta\epsilon_{Pblk}(t), \quad (8a)$$

where $\Delta\epsilon_{Pblk}(t)$ is equal to the change in the parasympathetic error bias from baseline $[\epsilon_{Pblk}(bsl)]$ to time t. Similarly, for the phasic parasympathetic contribution,

$$\Delta p'(t) = \phi_{Sblk}(t) - \phi_{Sblk}(bsl) + \Delta\epsilon_{Sblk}(t). \quad (8b)$$

There are several points of note in the above estimates. First, although the value of β may not be known, this constant cancels in the difference score derivation and hence does not enter into the analysis of phasic response. Second, Equations 8a and 8b reveal that the residual model estimates of sympathetic and parasympathetic control are biased by different error terms associated with blockade of the branch opposite to that being estimated. Finally, these estimates are biased not by the full magnitude of the respective blockade error term but only by the change in that bias from prestimulus levels to time t.

Subtractive model analysis. Alternative autonomic estimates can be derived from Equations 7a and 7b, reflecting the subtractive effects of autonomic blockade from the corresponding values in the unblocked condition (illustrated by the s'' and p'' arrows in Figure 1). Again, the absolute level of autonomic control is not of primary concern; of primary interest is the relative change (from baseline) in sympathetic control. The subtractive estimate of the sympathetic contribution to the functional state of the organ at any time (t) is a change score of the form

$$\Delta s''(t) = s''(t) - s''(bsl),$$

where $\Delta s'(t)$ represents the change in sympathetic control from prestimulus baseline ($s''[bsl]$) to time t. From the above equation and Equation 7a, the subtractive sympathetic estimate is

$$\Delta s''(t) = [\phi(t) - \phi_{Sblk}(t) - \epsilon_{Sblk}(t)] - [\phi(bsl) - \phi_{Sblk}(bsl) - \epsilon_{Sblk}(bsl)].$$

Simplifying this equation gives an estimate of the phasic sympathetic contribution:

$$\Delta s''(t) = \phi(t) - \phi_{Sblk}(t) - \phi(bsl) + \phi_{Sblk}(bsl) - \Delta\epsilon_{Sblk}(t), \quad (9a)$$

where $\Delta\epsilon_{Sblk}(t)$ is the change in the sympathetic error bias from baseline ($\epsilon_{Sblk}[bsl]$) to time t. Similarly, the subtractive estimate of parasympathetic contribution to the phasic response is derived from Equation 7b:

$$\Delta p''(t) = \phi(t) - \phi_{Pblk}(t) - \phi(bsl) + \phi_{Pblk}(bsl) - \Delta\epsilon_{Pblk}(t). \quad (9b)$$

These estimates represent the relative contributions of the autonomic branches to phasic response, as derived by the change in response after blockade of the target division. Again, these estimates are not biased by the full magnitude of the blockade error term but only by the difference in this bias from the prestimulus period to time t. In contrast to the residual estimates of Equations 8a and 8b, the corresponding subtractive estimates are biased by the error term associated with blockade of the target branch rather than with the opposite branch. This is an important distinction that permits derivation of indices of bias in the autonomic estimates.

An estimate of error (ϵ_{blk}). The residual estimates $\Delta s'$ and $\Delta p'$ and the subtractive estimates $\Delta s''$ and $\Delta p''$ represent alternative derivations that differ only in their error terms. In the absence of error bias, the computational formulas for the prime and double prime estimates are formally equivalent and must give identical results.[6] In the presence of error bias, however, the alternative residual and subtractive estimates will not agree, and the discrepancies between the prime and double prime values would be nonrandom. This result is apparent from residual model Equations in 8a and 8b and their corresponding subtractive estimates in Equations 9a and 9b. The s' and p' estimates of Equations 8a and 8b are positively biased by blockade error terms ($\Delta\epsilon_{Pblk}[t]$ and $\Delta\epsilon_{Sblk}[t]$, respectively). In contrast, the corresponding s'' and p'' estimates of Equations 9a and 9b are negatively biased by the error terms ($\Delta\epsilon_{Sblk}[t]$ and $\Delta\epsilon_{Pblk}[t]$, respectively). That this must be the case is apparent from Figure 1. The sum of the arrow vectors s' and p'' must equal the magnitude of the observed response. These two estimates are derived from the same (parasympathetic) blockade, and any bias that diminishes the estimate s' would necessarily increase the estimate p''. A similar symmetry exists for the p' and s'' estimates.

For either blockade condition, therefore, systematic errors bias the relevant s and p estimates to an equivalent extent but in opposite directions (positively biasing the prime estimates and negatively biasing the double prime estimates). Consequently, there is a necessary equality of the discrepancies between the s' and s'' estimates and between the p' and p'' estimates. Because the discrepancies between the prime and double prime estimates arise from biases related to both blockade conditions, they provide an aggregate index of these biases. The total range of bias from autonomic blockades is thus $\Delta s' - \Delta s'' = \Delta p' - \Delta p''$, and an index of the range of error around a central autonomic estimate would be

$$\pm\Delta\epsilon_{blk} = (\Delta s' - \Delta s'')/2 = (\Delta p' - \Delta p'')/2. \quad (10a)$$

Given the fact that the prime and double prime estimates of each autonomic branch are reciprocally biased by alternate blockades, the origins of this error bias are not derivable from the present analysis. In the absence of additional information, the average of the prime and double prime estimates would provide the best estimate of the true sympathetic and parasympa-

[6] This identity can be shown, for example, from the sympathetic estimates in Equations 8a and 9a. From Equation 8a and given a null error term, $\Delta s'(t) = \phi_{Pblk}(t) - \phi_{Pblk}(bsl)$. By substituting identities from Equation 2a, this becomes $\Delta s'(t) = [S(t) + \beta] - [S(bsl) + \beta]$, which simplifies to $\Delta s'(t) = S(t) - S(bsl)$. A similar identity can be shown for the subtractive model estimate of Equation 9a: $\Delta s''(t) = \phi(t) - \phi_{Sblk}(t) - \phi(bsl) + \phi_{Sblk}(bsl)$. Again, substituting identities from Equation 2b, $\Delta s''(t) = [\beta + S(t) + P(t)] - [P(t) + \beta] - [\beta + S(bsl) + P(bsl)] + [P(bsl) + \beta]$, which simplifies to $\Delta s''(t) = S(t) - S(bsl) = \Delta s''(t)$.

thetic contributions to phasic response. Hence, the estimated autonomic contributions at any point in time would be

$$\Delta s(t) = [\Delta s'(t) + \Delta s''(t)]/2 \pm \Delta\epsilon_{blk} \quad (10b)$$

$$\Delta p(t) = [\Delta p'(t) + \Delta p''(t)]/2 \pm \Delta\epsilon_{blk}. \quad (10c)$$

Simulations: Blockade biases and the error estimate. The error estimate ($\Delta\epsilon_{blk}$) offers an important index of bias in blockade studies. Quantitative model simulations were run to examine the sensitivity of $\Delta\epsilon_{blk}$ to potential sources of bias. From the peak response of the unbiased sample data (Figure 1B), simulated errors were added to the autonomic estimates to approximate each of the major classes of bias. Specifically, we emulated biases associated with a) interactions, b) indirect alterations of the unblocked branch, c) nonselective actions of the antagonists, and d) incomplete blockades. For the interaction simulation, we assumed reciprocal inhibitory influences among the autonomic branches, as is typical of the autonomic innervations of the heart (Hall & Potter, 1990; Levy, 1984; Manabe et al., 1991; Warner & Levy, 1989). We also assumed that these interactions would be eliminated by the respective autonomic blockers, because they otherwise do not bias autonomic estimates. For the simulation of indirect reflex alterations, we modeled various levels (0–50%) of blockade-induced attenuation of activity in the unblocked branch. This would be the pattern expected, for example, for the baroreceptor heart-period reflex. Formally, b) and c) are equivalent, each entailing blocker-induced alterations in reactivity of the unblocked branch, albeit by different mechanisms. Consequently, these will be considered together. Finally, simulations of incomplete blockades modeled the entire range of blockade (0–100%) both separately and jointly for the two branches.

Figure 2 shows the error estimate $\Delta\epsilon_{blk}$ as a function of various blockade-related biases. For all simulations, increasing the error bias introduced into the data yielded a progressive increase in the error estimate $\Delta\epsilon_{blk}$. Moreover, in each case, the value of $\Delta\epsilon_{blk}$ was exactly equal to the biases introduced into the autonomic estimates. This outcome is not adventitious but is a formal consequence of the derivations outlined above.

From the data of Figure 1B, the peak response in the unblocked condition is 75 ms, and the true contributions of the sympathetic and parasympathetic branches are 50 ms and 25 ms, respectively. Thus, if blockades were completely ineffective, the subtractive estimates ($\Delta s''$ and $\Delta p''$ from Equations 9a and 9b) would be 0, whereas the residual estimates ($\Delta s'$ and $\Delta p'$ from Equations 8a and 8b) would be equal to the magnitude of the unblocked response (75 ms). The overall estimated sympathetic and parasympathetic contributions (Δs and Δp of Equations 11a and 11b) would both be 37.5 ms rather than the true values of 50 and 25 ms. The large discrepancy between the prime and double prime estimates, however, yields a large bias estimate, and the error term ($\Delta\epsilon_{blk}$) would equal 37.5 ms (Equation 10a). The resulting summary statistics would be $\Delta s = 37.5 \pm 37.5$ ms and $\Delta p = 37.5 \pm 37.5$ ms. The error range in this case is as large as the mean estimates, and no meaningful conclusions could be derived from the blockade analysis.

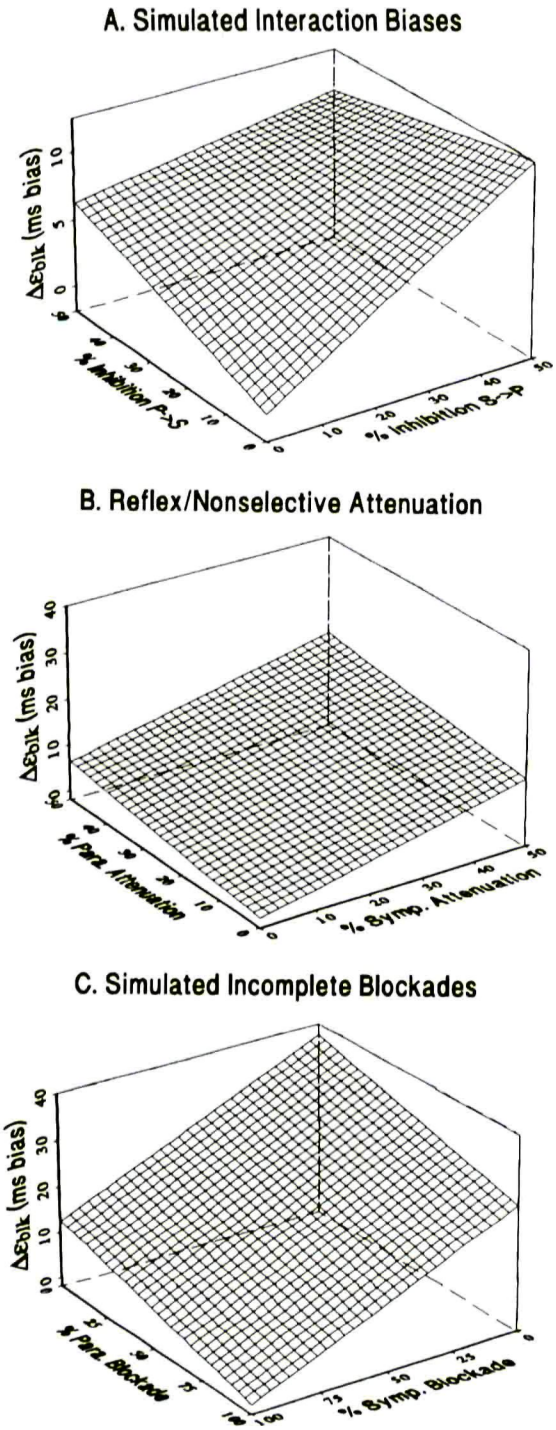

Figure 2. Values of the error term ϵ_{blk} (at the peak response) when systematic biases are added to the data from Figure 1B. A. Effects of reciprocal inhibitory interactions among the autonomic branches at the level of the heart. B. Effects of blockade-induced attenuation of the unblocked branch, due to reflexive adjustments or nonselective drug actions. C. Effects of varied degrees of completeness of the autonomic blockades.

Applications to empirical data. For illustration, the analysis of phasic response as outlined above was applied to the data of Quigley and Berntson (1990). This study examined the chronotropic responses of rats to a 1-s auditory stimulus, presented at two intensities (60 and 80 dB, SPL) designed to evoke orienting and defensive responses, respectively. Separate groups received the low- and the high-intensity stimuli, and each animal was tested in three separate counterbalanced sessions: one in the

unblocked state, one during sympathetic blockade (atenolol, 5 mg/kg), and one during parasympathetic blockade (scopolamine methyl nitrate, 0.1 mg/kg). The high-intensity stimulus evoked notable tachycardia, reminiscent of a defensive response (DR), whereas the low-intensity stimulus resulted in a predominant bradycardia, suggestive of an orienting response (OR).

Figure 3 (top panels) illustrates the heart period responses to the two stimuli in the unblocked condition (solid line) and after

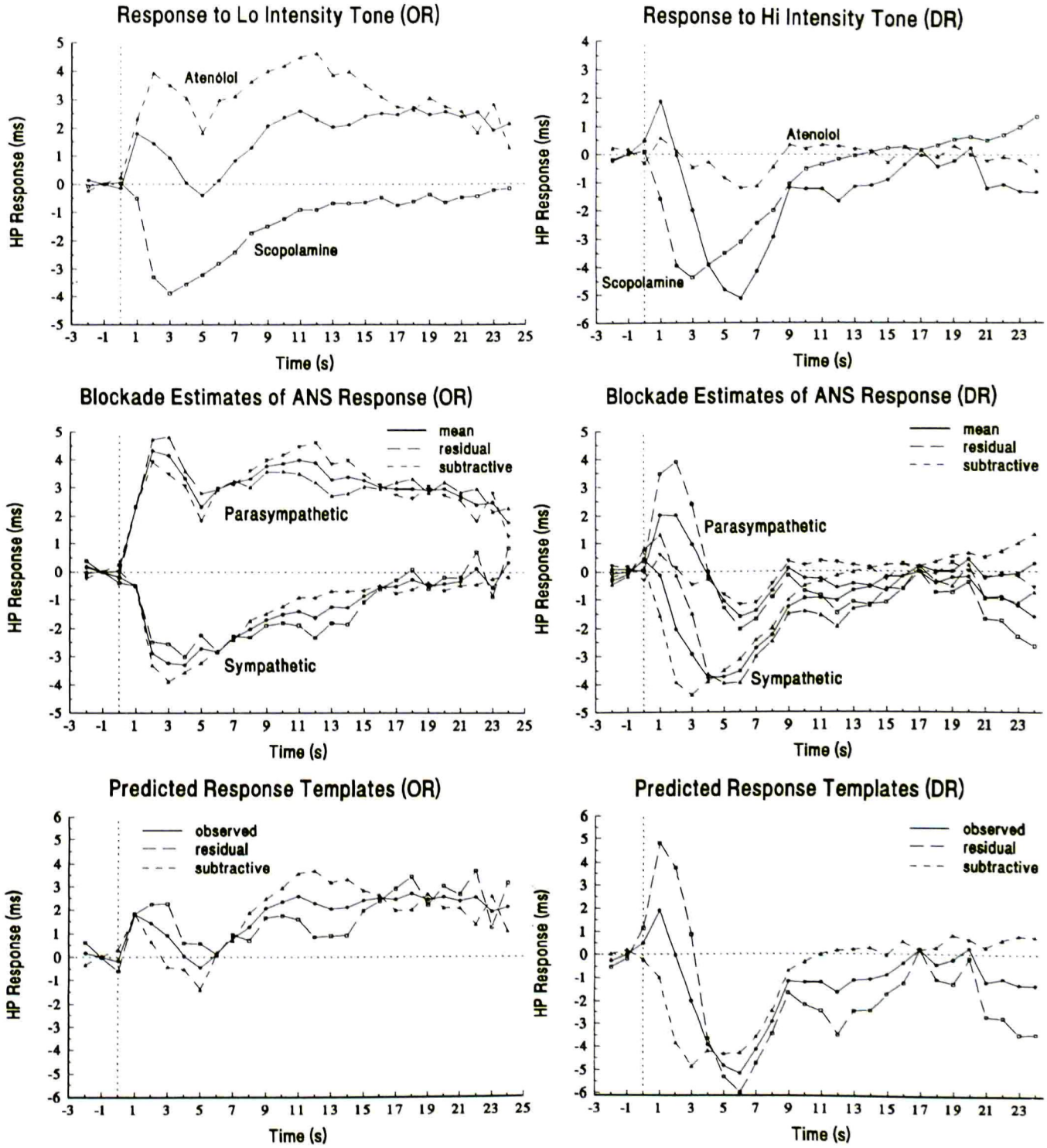

Figure 3. Heart period responses of rats to low- and high-intensity tones. Top: Observed responses after saline, sympathetic blockade (atenolol, 5 mg/kg), and parasympathetic blockade (scopolamine methyl nitrate, 0.1 mg/kg). Stimulus onset is indicated by the vertical dotted lines, and the prestimulus baseline is depicted by the horizontal dotted lines. Middle: Residual model (prime) and subtractive model (double prime) estimates of autonomic contributions to the phasic responses. Bottom: Observed (unblocked) response and predicted response templates based on the residual model estimates and the subtractive model estimates.

selective blockades of the autonomic divisions. The cardioacceleratory response to the high-intensity stimulus appeared to be mediated largely by an increase in sympathetic control; this response was largely eliminated by sympathetic blockade and was only minimally altered by parasympathetic blockade. In contrast, the decelerator response to the low intensity stimulus appeared to reflect coactivation of both autonomic branches. This response was eliminated by parasympathetic blockade, suggesting a vagal origin. Parasympathetic blockade not only eliminated the evoked bradycardia, however, but unmasked a notable cardioacceleratory response. The acceleratory response apparent under parasympathetic blockade likely reflected a concurrent sympathetic activation, which was obscured by the more potent vagal response in the unblocked condition. Consistent with this interpretation, sympathetic blockade yielded an increase in the magnitude of the decelerator response to the low-intensity stimulus. At a qualitative level, the results suggest that the cardioacceleratory response to the high-intensity stimulus was mediated largely by the sympathetic branch, whereas the decelerator response to the low-intensity stimulus appeared to be mediated by parasympathetic activation that was partially obscured by a concurrent sympathetic activation. Although these conclusions are reasonable, the empirical outcomes may be corrupted by biases introduced by autonomic blockades.

Further analysis of these data indicates that systematic biases from autonomic blockades are minimal. The middle panels of Figure 3 show the overall autonomic estimates (Δs, Δp), as derived from Equations 10b and 10c. Also illustrated are the separate $\Delta s'$, $\Delta s''$, $\Delta p'$, and $\Delta p''$ estimates that are inversely influenced by biases inherent in the autonomic estimates. In general, the error bias was modest, relative to the magnitudes of the responses of the autonomic branches. These findings increase confidence in the interpretations of the blockade data. Larger error values were apparent, however, in the early poststimulus period of the DR. Although the overall parasympathetic estimate Δp suggests a transient parasympathetic response to the high-intensity stimulus, this interpretation must be tempered by the large error range in that portion of the function.

As a related test of the validity of inferences derived from blockade data, a predicted template of the unblocked response can be derived from the prime ($\Delta\phi' = \Delta s' + \Delta p'$) and double prime ($\Delta\phi'' = \Delta s'' + \Delta p''$) estimates. The chronotropic responses observed in the unblocked conditions are illustrated, along with the predicted response templates, in the bottom panels of Figure 3. Any bias in the autonomic estimates, introduced by blockade confounds, would yield predicted response templates that systematically deviate from the response observed in the unblocked condition. Although discrepancies are apparent between the predicted and observed functions of Figure 3, the overall pattern of response of the two autonomic branches is clear. In fact, the moderate size of the discrepancies were especially notable because the individual drug conditions were run in separate sessions on separate days.

Impact of basal values. The analysis of phasic response is independent of absolute level of functional organ state. However, selective blockades may substantially alter the basal functional level of the organ (Figure 1B). An issue thus arises as to the potential impact of this level shift on organ response. This issue may not be a major concern for the chronotropic control of the heart as long as the chronotropic metric is heart period. Sympathetic and parasympathetic outflows show an essentially linear relationship with heart period across the functional chronotropic range (Berger et al., 1989; Berntson, Quigley, Fabro, & Cacioppo, 1992; Dexter et al., 1989; Koizumi, Terui, & Kollai, 1985; Lang & Levy, 1989; Parker, Celler, Potter, & McCloskey, 1984; Versprille & Wise, 1971). Consequently, the heart period manifestation of a given autonomic response would be equivalent regardless of the basal chronotropic level at which it is expressed. The prime and double prime autonomic estimates illustrated in Figure 3 (middle panels) were derived from different blockade conditions and hence were subject to basal shifts in the opposite direction. The relatively small discrepancies between these estimates indicate that basal shifts did not introduce appreciable error into the estimates. It nevertheless remains possible in some circumstances that level shifts associated with a given blockade may increase the functional state of the organ toward its physiological limit and constrain subsequent incremental responses. This shift would bias only the estimates derived from one blockade condition, because the alternative blockade would not be expected to yield similar shifts and may in fact shift the functional state in the opposite direction. Hence, constraints imposed by physiological maxima yield discrepancies between the prime and double prime estimates and would thus appropriately appear in the error estimate ($\Delta\epsilon_{blk}$). Although the present analysis does not assume linearity in autonomic control of the heart, nonlinearities can bias estimates and thus are treated like any other source of bias.

Analysis of Basal Autonomic Tone

If a dual blockade condition is included in the design, the general principles outlined above can also be applied to evaluate the absolute levels of autonomic control of chronotropic state. The application of selective sympathetic and parasympathetic blockades in the analysis of tonic autonomic control yields four primary sets of data: a) the basal level in the unblocked condition (ϕ in Figure 1A), b) the level under sympathetic blockade (ϕ_{Sblk}), c) the level under parasympathetic blockade (ϕ_{Pblk}), and d) the organ state under dual sympathetic and parasympathetic blockade (ϕ_{SPblk}). These values permit both residual and subtractive estimates of autonomic control from Equations 6a and 6b and Equations 7a and 7b, respectively.

Residual model estimates of autonomic control are represented by the arrows s' and p' in Figure 1A. These values represent the absolute tonic contributions of sympathetic and parasympathetic control to basal chronotropic state, referenced to the zero point of autonomic control (β). Subtractive model estimates of sympathetic and parasympathetic control are depicted by the s'' and p'' arrows in Figure 1A, as specified in Equations 7a and 7b. As documented above, the prime and double prime estimates are equivalent in the absence of error bias. Again, blockade-induced biases differentially impact on the prime and double prime estimates, and the difference between these estimates defines the magnitude of bias. The error term for biases in absolute autonomic estimates parallels that for the phasic response (Equation 10a):

$$\epsilon_{blk} = (s' - s'')/2 = (p' - p'')/2. \qquad (11a)$$

Similarly, the equations for the absolute sympathetic and parasympathetic branches parallel the values for phasic responses (Equations 10b and 10c):

$$s = (s' + s'')/2 \pm \epsilon_{blk} \quad (11b)$$

$$p = (p' + p'')/2 \pm \epsilon_{blk}. \quad (11c)$$

β and its error of estimate. β represents the intrinsic functional state in the absence of autonomic control. Equation 2c provides an estimate of this intrinsic state from dual blockades ($\beta_0 = \phi_{SPblk}$). Alternative estimates of intrinsic state can be derived from Equation 1c ($\beta = \phi - S - P$) together with Equations 6a and 6b and 7a and 7b, yielding a total of three estimates:

from Equation 2c: $\beta_0 = \phi_{SPblk}$

from Equations 6a, 6b: $\beta' = \phi - s' - p'$

from Equations 7a, 7b: $\beta'' = \phi - s'' - p''$.

In the absence of error, s' equals s'', p' equals p'', and hence β' equals β''. Moreover, in the absence of error, both β' and β'' are equal to β_0. Because the error terms for these estimates are different, however, error bias can lead to discrepancies among these estimates. The error terms for these alternative estimates (from Equations 2c, 6a and 6b, and 7a and 7b) are

error bias in β_0: $+ \epsilon_{SPblk}$

error bias in β': $-\epsilon_{Pblk} - \epsilon_{Sblk} + 2\epsilon_{SPblk}$

error bias in β'': $+\epsilon_{Sblk} + \epsilon_{Pblk}$.

As is apparent from these error terms, the β_0, β', and β'' estimates of β are differentially biased by error. Moreover, the β' and β'' estimates are symmetrically distributed about β_0, as revealed by the differences in error bias between these estimates and β_0 (from the above equations):

$$\beta' - \beta_0: - \epsilon_{Pblk} - \epsilon_{Sblk} + \epsilon_{SPblk}$$

$$\beta'' - \beta_0: + \epsilon_{Pblk} + \epsilon_{Sblk} - \epsilon_{SPblk}.$$

The prime and double prime estimates of β are inversely affected by biases associated with blockades. The β_0 estimate (derived from dual blockade) is biased only by incomplete blockades (ϵ_{SPblk})[7] and can never be more biased than the prime and double prime estimates. Moreover, any error bias that yields a discrepancy between β_0 and β' would yield an equal but opposite discrepancy between β_0 and β''. The difference between the β' and β'' estimates, therefore, provides an index of error bias for the β_0 estimate:

$$\epsilon_\beta = (\beta' - \beta'')/2. \quad (12)$$

Estimates of the individual autonomic branches are inversely biased by blockades, and the discrepancies between the prime and double prime estimates provide an index of error (ϵ_{blk}, Equation 11a) for the autonomic estimates. β' and β'', however, are derived from estimates of both autonomic branches and hence are biased by errors in both estimates. This bias is apparent in the error terms for β' and β'' given above. Consequently, the following identity holds:

$$\epsilon_\beta = 2\epsilon_{blk} = s' - s'' = p' - p''$$

Effects of incomplete blockades. In the analysis of phasic response, incomplete blockades of the individual branches have opposite effects on the prime and double prime autonomic estimates and hence appear as biases in the associated error term ($\Delta\epsilon_{blk}$). In contrast to the phasic analysis, the residual estimates of absolute levels (Equations 6a and 6b) include an additional error component (ϵ_{SPblk}) that represents the effects of incomplete blockades in the dual blockade condition. This error component does not appear in the subtractive estimates (Equations 7a and 7b) because the subtractive values are referenced to baseline rather than intrinsic heart period. For incomplete blockades in tonic analyses, the symmetry in the effects of bias breaks down, although it continues to hold for other classes of bias and for incomplete blockades in phasic analyses. This breakdown is due to the fact that incomplete blockades bias not only the heart period levels in the single blockade conditions (ϕ_{Sblk}, ϕ_{Pblk}) but the β_0 estimate that serves as the zero point against which the ϕ_{Sblk} and ϕ_{Pblk} levels are assessed (see Equations 6a and 6b). Because ϵ_{SPblk} can not be directly indexed, incomplete blockades may not be detectable in the analysis of absolute levels (although incomplete blockade biases would be detectable in the analysis of level changes). Fortunately, the completeness of blockades can be explicitly tested.

Documenting complete blockades. For the analysis of phasic response, incomplete blockades inversely bias the prime and double prime estimates and hence appear in the error bias estimate $\Delta\epsilon_{blk}$. Because incomplete blockades are not detectable in the analysis of absolute levels of autonomic control, the effectiveness of blockades should be assured by dose–response studies or by explicit test. One approach to establishing the effectiveness of blockades is to test the heart period effects of potent autonomic reflexes, which should be largely eliminated by dual autonomic blockade condition. Phenylephrine and/or nitroprusside, for example, should be incapable of evoking baroreflex responses under dual blockade. For human subjects, a postural manipulation such as sitting versus standing can be included in the design, and the normal reflexive orthostatic adjustments in heart period should be prevented by dual blockade. That is, under dual blockade, the estimate of intrinsic heart period (β_0) should be stable across experimental conditions and not contaminated by autonomic adjustments to postural change or other autonomically evocative conditions.

Simulations: Incomplete blockades. The transparency of incomplete blockades in analyses of absolute levels of autonomic control can be demonstrated by simulations. Input data were the bias-free data of Figure 1A, to which biases were added to represent the effects of incomplete blockades. Various levels of blockade were simulated (0–100%), both separately and jointly for the two autonomic branches. For a given incomplete blockade, we modeled a proportional reduction in the effects of this blockade condition on heart period. Reference to Figure 1A, for example, reveals that a partially incomplete sympathetic blockade would yield a decrease in the value of ϕ_{Sblk} and an equivalent increase in the value of ϕ_{SPblk}. If sympathetic blockade

[7] Because all autonomic control is precluded under effective dual blockade, chronotropic manifestations of interactions, nonselective receptor actions of the blockers, and indirect actions would all be null.

were completely ineffective, ϕ_{Sblk} would equal baseline heart period ($\phi[bsl]$) and ϕ_{SPblk} would equal the level under parasympathetic blockade (ϕ_{Pblk}).

Although these simulations yielded widely different sympathetic, parasympathetic, and β estimates, in no case was a discrepancy observed between the prime and double prime estimates. Representative results are illustrated in Table 2 for the 0%, 50%, and 100% blockade conditions. Progressive reductions in the effectiveness of the blockades gave progressively smaller autonomic estimates, which assume the value of zero when blockades are completely ineffective. In contrast, estimates β of became progressively larger, equalling baseline heart period with completely ineffective blockades. A final important feature is the effect of postural manipulations on estimates of β. Intrinsic heart period is a relatively stable individual characteristic (Jose, Stitt, & Collison, 1970, see also Berntson et al., 1993) and should not be altered by moderate postural manipulations. Because autonomic control was effectively precluded in the 100% blockade condition, no differences in the β_0 estimate were apparent across postures. With progressively less effective blockades, however, the β estimates changed across postures, and this difference becomes larger the less effective the blockade. Indeed, as discussed above, the stability of β estimates across posture or other evocative conditions can be employed as a specific test of the effectiveness of blockades.

Analysis of empirical data. The analytic approach outlined above was applied to the estimates of tonic autonomic contributions to chronotropic state as a function of postural state. Data were derived from studies by Nyberg (1981), Saul et al. (1991), and Robinson, Epstein, Beiser, and Braunwald (1966), in which subjects were tested under unblocked conditions after selective sympathetic (propranolol, 0.25–0.40 mg/kg) or parasympathetic (atropine, 0.03–0.04 mg/kg) blockades and after dual blockade. Basal heart period measures were obtained for each subject while supine and while standing (0° and 80° tilt). The Saul et al. (1991) study employed separate groups for the single blockade conditions, whereas the other two studies were entirely within subjects and included a sitting condition (45° tilt). Estimates of autonomic control and β were derived as outlined above.

Results of these studies are detailed in Table 2. First, blockades appeared to be relatively complete as indicated by the general stability of β_0 across postural manipulations (Ms = 606–636 ms), although some variation was apparent. Second, discrepancies between the prime and double prime autonomic estimates, and hence the error bias (ϵ_{blk}), were generally modest, although they became somewhat larger in the supine condition. Third, by formal necessity, a) the differences between s' and s'' are equal to those between p' and p'', b) these differences are exactly half the difference between β' and β'', and c) the values of ϵ_β are precisely twice those of the corresponding ϵ_{blk}. Finally, the expected changes in autonomic control were apparent across postures. Relative to the supine posture, standing was associated with an increase in sympathetic control and decrease in parasympathetic control as revealed by the s and p estimates. Moreover, these differences were larger than error bias terms (ϵ_{blk}). The mean decrement in parasympathetic control from supine to standing was 146 ms, relative to error estimates of ±3 and ±32 for these postural conditions. The mean increment in sympathetic control was 62 ms, relative to the same error terms.

Applications and Derivation of a Validity Metric

Interpretations of blockade data are dependent on the validity of the derived autonomic estimates, which are subject to multiple potential biases. The residual and subtractive models outlined above provide quantitative metrics of sympathetic and

Table 2. *Sympathetic and Parasympathetic Contributions to Chronotropic State as a Function of Posture*

Source	Posture	s'/s''	s	p'/p''	p	ϵ_{blk}	β'/β''	β_0	ϵ_β
Simulations									
100% blockade	Standing	−100/−100	−100	400/400	400	0	600/600	600	0
50% blockade	Standing	−50/−50	−50	200/200	200	0	750/750	750	0
0% blockade	Standing	0/0	0	0/0	0	0	900/900	900	0
100% blockade	Sitting	−160/−160	−160	300/300	300	0	600/600	600	0
50% blockade	Sitting	−80/−80	−80	150/150	150	0	670/670	670	0
0% blockade	Sitting	0/0	0	0/0	0	0	740/740	740	0
Empirical studies									
Nyberg (1981)	Standing	−131/−133	−132	334/332	333	±1	586/588	587	±2
Saul et al. (1991)	Standing	−145/−177	−161	264/232	248	±16	613/677	645	±32
Robinson et al. (1966)	80° tilt	−191/−181	−171	314/304	309	±5	629/619	624	±10
Means		−161/−157	−159	324/318	321	±3	608/604	606	±6
Nyberg (1981)	Sitting	−130/−88	−109	415/457	436	±21	630/588	606	±42
Robinson et al. (1966)	45° tilt	−157/−136	−147	400/421	411	±11	653/632	643	±21
Means		−144/−112	−128	408/439	424	±16	642/610	626	±32
Nyberg (1981)	Supine	−103/−54	−79	446/495	471	±25	674/576	625	±49
Saul et al. (1991)	Supine	−82/−164	−123	402/320	362	±40	550/714	632	±80
Robinson et al. (1966)	0° tilt	−58/−118	−88	598/538	568	±30	592/712	652	±60
Means		−81/−112	−97	482/451	467	±32	605/667	636	±63

Note: All values are expressed in milliseconds of heart period. For illustrated simulations, effectiveness of the blockade was equivalent for the two autonomic divisions. Starting data for the simulations were the unbiased error-free values in Figure 1A, to which biases were added simulating results of various degrees of blockade effectiveness.

parasympathetic control that are subject to directionally opposite biases by selective blockades of the two autonomic branches. The discrepancies between these model estimates thus offer an index of systematic error bias in blockade studies and permit an assessment of validity of the autonomic estimates. The derivation of autonomic estimates outlined above is similar to quantitative methods previously described (Lin & Horvath, 1972; Stemmler et al., 1991). The primary contribution of the present approach is the application of both residual and subtractive model estimates to yield an unbiased estimate of autonomic chronotropic control and to derive quantitative indices of validity. The equations presented in Table 1 are formally derived and permit the estimation of the relative contributions of the autonomic branches to both tonic chronotropic state and phasic response. For tonic estimates, a dual blockade condition is necessary to estimate β. Although β is employed in the formal derivation of the equations for phasic response, this term cancels out (see derivation of Equation 8a), permitting the estimation of the autonomic contributions to phasic response from single blockades alone.[8]

Standard statistical approaches can be employed to address the stochastic reliability of the estimates of sympathetic and parasympathetic contributions to tonic chronotropic state and phasic response of the heart. Inferential statistics do not address the issue of validity, however, and biases in the autonomic estimates can lead to specious interpretations, even when statistical significance is obtained. Systematic biases can contribute to statistical significance even in the absence of a true experimental effect. The error terms ($\Delta\epsilon_{blk}$ and ϵ_{blk}) capture biases from autonomic blockade and hence provide validity indices for the phasic and tonic autonomic estimates. The error terms differ from the standard deviation, the standard error, or other metrics of dispersion in that $\Delta\epsilon_{blk}$ and ϵ_{blk} index systematic bias rather than random variance. In contrast to reliability estimates such as the standard error, $\Delta\epsilon_{blk}$ and ϵ_{blk} are not decreased by increasing sample size. Rather, they represent indices of inherent validity of the estimates.

Descriptive and inferential metrics. By representing the inherent ambiguity in autonomic estimates, the error terms $\Delta\epsilon_{blk}$ and ϵ_{blk} specify the fundamental resolution of the autonomic metrics. Hence, they constitute basic descriptive statistics that, along with indices of distribution or variance, can facilitate interpretation of autonomic estimates. Specification of the mean autonomic estimates ($\Delta s/\Delta p$ or s/p) together with error biases ($\Delta\epsilon_{blk}$ or ϵ_{blk}) inherent in these estimates would more fully characterize the results of blockade analysis.

Inferential statistics can be used to evaluate specific autonomic effects or experimental contrasts. Although significance implies statistical reliability, it does not insure validity of the autonomic estimates or their interpretation. A useful metric in judging the validity of significant experimental effects is the ratio of the size of the effect of interest to the size of the effect plus error. This statistic v_δ represents a coefficient of validity for experimental effects:

$$v_\delta = \frac{|\textit{effect size}|}{|\textit{effect size}| + |\textit{error bias}|}, \tag{13}$$

where *effect size* is the magnitude of the experimental effect and *error bias* is the magnitude of systematic biases in the autonomic estimates. The validity coefficient (v_δ) ranges from 1 in the absence of error bias to 0 when all the variance is error. If the experimental contrast were between an autonomic estimate and some hypothesized level (such as 0), the effect size would be the value of the autonomic estimate minus the hypothesized level (e.g., $s - 0$), and the error bias would be ϵ_{blk} (for absolute estimates) or $\Delta\epsilon_{blk}$ (for the phasic case). If the experimental interest were in the relative difference in activity of an autonomic branch across groups or under two distinct experimental conditions, the effect size would be the difference in the two autonomic estimates. Because bias is inherent in each of the estimates, the most conservative value of the *error bias* term of Equation 13 would be the sum of the error terms for the two estimates.[9]

At a value of $v_\delta = 0.5$, the magnitude of error would equal to the magnitude of the experimental effect. As a minimal rule, statistically significant effects associated with a validity coefficient of ≤ 0.5 should not be considered valid for purposes of interpretation. For values >0.5, the general confidence in interpretations would of course increase with increasing values of v_δ. This validity coefficient may be useful even if experimental effects fail to attain significance. If results were insignificant and the validity coefficient for the experimental effect were large, it would suggest a valid disconfirmation of the hypothesis. In contrast, a small validity coefficient under the same circumstances raises the possibility that true experimental effects might be obscured by error bias. Although this would not salvage a statistically unreliable outcome, it would caution against a definitive rejection of the hypothesized effect. In the latter case, additional analysis or study of the hypothesized relationship may be warranted.

The blockade data presented above yielded relatively low error terms and high validity coefficients. The present analyses are especially important, however, when this is not the case. The availability of quantitative measures of bias can preclude inappropriate interpretations of blockade data and may guide subsequent studies of the origins of these biases.

Overview and Caveats

The methods outlined above provide a quantitative approach to the analysis of autonomic control through selective pharmacological blockades. Although previous studies have employed residual and/or subtractive procedures (Lin & Horvath, 1972; Stemmler et al., 1991), the important aspect of the present

[8]For the analysis of phasic response, identical results will be obtained either from the phasic equations or by deriving a difference score based on the tonic equations. The appreciable advantage of the phasic equations is due to the fact that a dual blockade condition is not necessary when only phasic estimates are needed.

[9]For fixed effects designs, where comparisons are being made across groups (e.g., gender or strain), between-group differences could exist in the origins of error bias. Hence, the sum of the error terms would be the proper value for the error bias in Equation 13. In some cases however, especially in within-subjects designs, blockade biases may be common across experimental conditions. In this case, estimates under both experimental conditions would likely be biased in the same direction, and the more appropriate error term in Equation 13 may be the larger rather than the sum of the error terms of the two conditions. Because this approach is less conservative however, it should probably be used only with explicit justification.

approach is the quantitative application of both models to derive an index of error bias. For the analysis of phasic response, any bias from a given blockade necessarily exerts opposite effects on the prime and double prime estimates derived from that blockade condition, including the effects of incomplete blockades and even potential nonautonomic drug effects on the myocardium. As with most methods, however, the blockade approach is not without limitations. It is possible, for example, that a sympathetic blocker could spuriously enhance the apparent parasympathetic response, and the parasympathetic blocker could spuriously attenuate the sympathetic response. To the extent to which these effects are equal, they would bias the results but their contributions to the error term would cancel (i.e., the prime and double prime estimates would be comparable). Although possible, this situation would be most unusual from a mechanistic standpoint. In any event, the ability to quantitatively detect the most probable sources of bias in blockade studies confers considerable advantages to the approach outlined above.

If a dual blockade condition is included in the experimental design, absolute as well as relative levels of autonomic control can be derived. This analysis requires estimation of the intrinsic functional state of the organ, as derived from dual autonomic blockades. In this case, biases introduced by incomplete blockades do not manifest in the error term. Consequently, effectiveness of the autonomic blockades must be assured through other methods. An additional source of bias must also be considered in estimating absolute autonomic levels. The present analysis assumes a fixed, stable intrinsic functional state of the organ (β). Although individual differences have been demonstrated in intrinsic heart period, β has been reported to show a general within-subjects stability over time (Jose et al., 1970). The stability of intrinsic heart period is context dependent, however, because β can be transiently altered, for example, by changes in temperature or aerobic output (Berntson et al., 1993; Bolter & Atkinson, 1988; Ribeiro, Ibanez, & Stein, 1991; Sutton, Cole, Gunning, Hickie, & Seldon, 1967). Hence, care must be taken in interpreting blockade studies that entail notable differences in variables, such as aerobic output, that could alter β across experimental conditions. Fortunately, experimentally induced shifts in β are detectable by the change in the β_0 estimate (derived from dual blockade) across conditions. An observed shift in β_0, however, must be differentiated from the potential effects of incomplete blockades.

Despite inherent limitations, blockade studies constitute an important approach to quantifying the relative contributions of the autonomic branches to chronotropic control. The analytic methods presented above represent a significant advance over traditional approaches in permitting specification of systematic bias in autonomic estimates and providing quantitative validity indices to facilitate interpretations.

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