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β-ADRENOCEPTOR BLOCKING DRUGS AND ISOPRENALINE: CENTRAL EFFECTS ON CARDIOVASCULAR PARAMETERS

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1 The central hypotensive activity of (+)- and (−)-propranolol (100 μg), pindolol (100 μg) and isoprenaline (1 and 4 μg) injected intracerebroventricularly (i.c.v.) was studied in rats anaesthetized with urethane and chloralose. Blood pressure, cardiac output and heart rate were measured; systolic stroke volume and peripheral vascular resistance were calculated.

2 (+)- and (−)-Propranolol and pindolol induced a fall of blood pressure but (+)-propranolol was less active. The heart rate was reduced more by (−)-propranolol than by (+)-propranolol or (−)-pindolol. The decrease of systolic stroke volume was greater for (−)-propranolol and pindolol than for (+)-propranolol. Peripheral vascular resistance was reduced to the same level but with different time courses, (−)-propranolol having a longer effect than (+)-propranolol and pindolol.

3 Isoprenaline induced a hypotensive effect, while cardiac output and heart rate increased; the systolic stroke volume remained stable but peripheral vascular resistance was significantly decreased.

4 These results suggest that different central regulatory centres are involved in the control of cardiac function and peripheral vascular tone.

Introduction

The intracerebroventricular (i.c.v.) injection of β-adrenoceptor blocking agents induces bradycardia in cats (Kelliher & Buckley, 1970), dogs (Srivastava, Kulshrestha, Singh & Bhargava, 1972), rabbits (Reid, Lewis, Myers & Dollery, 1974) and rats (Ito & Schanberg, 1974), whilst under the same experimental conditions, β-adrenoceptor stimulating drugs, especially isoprenaline, provoke tachycardia in dogs (Bhargava, Mishra & Tangri, 1972) cats (Gagnon & Melville, 1967) and rabbits (Toda, Matsuda & Shimamoto, 1969; Reid *et al.*, 1974; Day & Roach, 1974a).

However, β-blocking drugs and isoprenaline, when injected separately i.c.v., induce a fall in blood pressure (Toda *et al.*, 1969; Kelliher & Buckley, 1970; Schmitt & Fenard, 1971; Srivastava *et al.*, 1972; Reid *et al.*, 1974).

These centrally mediated effects indicate that the antagonism between β-blocking and β-stimulating drugs is apparent only on heart rate. Srivastava *et al.* (1972) and Ito & Schanberg (1974) suggested that the cardiac and vascular regulating centres were under different control mechanisms. In order to obtain more information concerning this hypothesis, the effects of i.c.v. injections of (−)-propranolol, (+)-propranolol, pindolol and isoprenaline on blood pressure, heart rate, cardiac output and calculated peripheral vascular resistance were studied in anaesthetized rats.

Methods

Male Sprague Dawley SPF rats weighing 270 to 350 g were used. Anaesthesia was induced with pentobarbitone (60 mg/kg i.p.) and continued by a perfusion (1.2 ml/h) through the pudendal vein of isotonic sodium chloride solution containing 10% urethane and 0.8% α-chloralose.

Measurement of cardiovascular parameters

The femoral artery was cannulated and blood pressure (mmHg) was recorded with a pressure transducer. Heart rate (beats/min) was measured from the electrocardiographic recording obtained from D_2 derivation. Cardiac output was measured by Fegler's thermodilution method (1954) as modified by Evonuk, Jmig, Greenfield & Firstein (1961), and expressed in ml min^{-1} kg^{-1} body weight. Peripheral vascular resistance (in PRU units) was obtained from the ratio between blood pressure and cardiac output as formulated by Green, Repela & Conrad (1964).

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Stroke volume in ml was obtained by dividing cardiac output by heart rate.

Testing sequence and i.c.v. injection

The parameters were measured four times at 3 min intervals before administration of drugs; 10 µl of each drug solution used was injected into the lateral cerebral ventricle in 1 min according to the technique described by Noble, Portmann & Axelrod (1967). Blood pressure, heart rate and cardiac output were subsequently measured every 5 min for 30 min.

Drugs

(+)-Propranolol hydrochloride and (−)-propranolol hydrochloride were purchased from ICI laboratories; pindolol from Sandoz AG; (+)-isoprenaline hydrochloride was obtained from Sigma. (−)-Propranolol, (+)-propranolol and pindolol were used at a dose of 100 µg. The drugs were dissolved in 0.9% w/v NaCl solution (saline). Pindolol was dissolved by the addition of 0.1 ml 1 N HCl. The pH was then adjusted to 7.3 with 5% w/v sodium bicarbonate solution. To dissolve isoprenaline, the Winthrop laboratories' solvent was used (60% sodium lactate solution 0.3 ml, lactic acid 0.01 ml, NaCl 0.7 g, Na_2SO_3 0.1 g; distilled water was added to a final volume of 100 ml.)

Freshly prepared solutions were used. Control rats received saline.

Data analysis

Results obtained on groups of six rats are expressed as the mean ± s.e. mean. The basal values of the different parameters were obtained by calculating the mean of the results at 0, 3, 6 and 9 min. Statistical analyses were performed with Student's t test for paired comparisons. A value of $P < 0.05$ was considered significant.

Results

Control rats

Saline or isoprenaline solvent had no significant effects on the basic level of the cardiovascular parameters. The decrease in blood pressure was 3 ± 2 mmHg, with an initial value of 116 ± 9 mmHg for saline and 3 ± 2 mmHg for the isoprenaline solvent (initial value: 108 ± 5 mmHg) after 20 min. Heart rate, cardiac output, systolic stroke volume and peripheral vascular resistance did not change after i.c.v. injection of the two solvents (Figures 1 and 2).

Effects of drugs on cardiovascular parameters

Blood pressure Isoprenaline (i.c.v.) at doses of 1 and 4 µg induced a significant hypotensive effect (Figure 1a). A dose of 1 µg produced only a brief fall in blood pressure, not exceeding 8 ± 3 mmHg (initial value: 114 ± 5 mmHg). Injection of the higher dose was followed by a longer period of hypotension, with a maximal decrease of 22 ± 4 mmHg (−22%) after 10 min, and 23 ± 4 mmHg (−23%) after 30 min (initial value: 100 ± 3 mmHg) ($P < 0.01$).

Similar falls in blood pressure were obtained with (−)-propranolol and pindolol (Figure 2a). Hypotension began during the injection, reached 43 ± 6 mmHg (−43% of the initial blood pressure which was 99 ± 4 mmHg) after 20 min with (−)-propranolol, 38 ± 3 mmHg (−36% of the initial value which was 107 ± mmHg) with pindolol and lasted more than 30 min ($P < 0.01$). The dextroisomer of propranolol was less active, the fall in blood pressure being 29 ± 5 mmHg (−32%) after 10 min. Blood pressure then increased slightly but remained lower than the initial value (92 ± 6 mmHg). The hypotension induced by (+)-propranolol was significantly less than that induced by (−)-propranolol and pindolol after the tenth minute ($P < 0.05$).

Cardiac output Isoprenaline 1 µg did not induce significant variations in cardiac output (Figure 1b) but 4 µg significantly increased it during 20 min by 29 ± 8 ml min^{-1} kg^{-1} (+12%; initial value: 242 ± 21 ml min^{-1} kg^{-1}).

Intracerebroventricular injection of all three β-blocking drugs caused, for the first 10 min after the injection, a significant decrease in cardiac output (Figure 2b). After 10 min it was reduced by 47 ± 13 ml min^{-1} kg^{-1} (−20% of the initial value which was 240 ± 25 ml min^{-1} kg^{-1}), by 69 ± 11 ml min^{-1} kg^{-1} (−34%; initial value: 200 ± 20 ml min^{-1} kg^{-1}) and by 61 ± 17 ml min^{-1} kg^{-1} (−27%; initial value: 229 ± 38 ml min^{-1} kg^{-1}) for (+)-propranolol, (−)-propranolol and pindolol respectively. The decrease in cardiac output remained constant with (−)-propranolol and pindolol ($P < 0.01$), but progressively returned to its initial value after 20 min with (+)-propranolol.

Peripheral vascular resistance The lower dose of isoprenaline induced a steady lowering of the peripheral vascular resistance (PVR), 0.039 ± 0.015 PRU (−8%, initial value: 0.464 ± 0.047 PRU) after 20 min. With the higher dose, the fall was more severe and reached 0.116 ± 0.21 PRU (−20%, initial value: 0.430 ± 0.043 PRU) after 20 min (Figure 1c). The decrease of the peripheral vascular resistance remained constant throughout the experiment and accounted for the observed hypotension ($P < 0.01$).

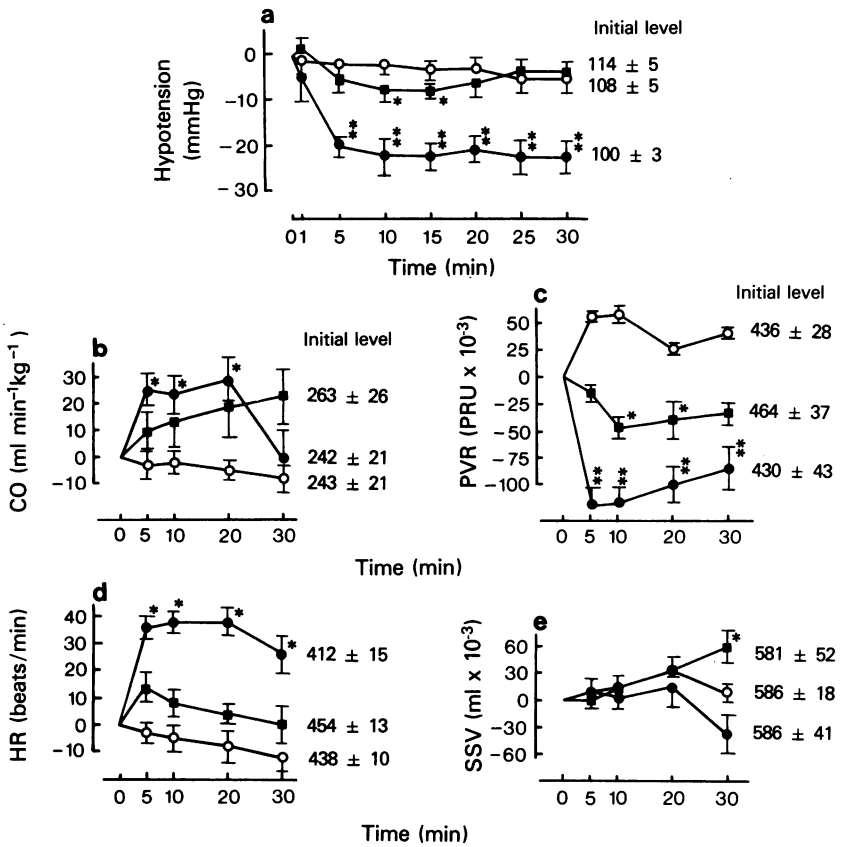

Figure 1 Changes in cardiovascular parameters after intracerebroventricular injection of isoprenaline: (○) control rats; (■) isoprenaline 1 μg; (●) isoprenaline 4 μg. (a) Hypotension; (b) cardiac output (CO); (c) peripheral vascular resistance (PVR); (d) heart rate (HR); (e) systolic stroke volume (SSV). Blood pressure is given in mmHg; cardiac output in ml min^{-1} kg^{-1} body wt.; peripheral vascular resistance units obtained from the ratio between blood pressure and cardiac output; stroke volume is expressed in ml per systole. Differs significantly from the initial value by $*P < 0.05$; $**P < 0.01$.

With β-blocking drugs we observed a decrease of peripheral vascular resistance which reached the same level for the three β-blocking agents during the first 10 min. After 10 min, the decrease was 0.058 ± 0.010 PRU (−15% of initial value which was 0.394 ± 0.032 PRU), 0.070 ± 0.025 PRU (−14%, initial value: 0.518 ± 0.045 PRU) and 0.050 ± 0.019 PRU (−10%, initial value: 0.513 ± 0.082 PRU) for (+)-propranolol, (−)-propranolol and pindolol respectively. Peripheral vascular resistance continued to decrease until the end of experiment with (−)-propranolol (0.095 ± 0.017) PRU (−19%) and remained at the same level with (+)-propranolol (0.051 ± 0.025 PRU (−13%) at the thirtieth min ($P < 0.05$).

With pindolol, however, peripheral vascular resistance at the thirtieth minute was not significantly different from the initial value.

Heart rate Isoprenaline 1 μg did not induce significant variations in the heart rate but 4 μg significantly ($P < 0.05$) increased heart rate by 38 ± 15 beats/min (+9%, initial value: 412 ± 15 beats/min) after 20 min (Figure 1d).

The bradycardia induced by 100 μg of (−)-propranolol was significantly greater than that observed with the other drugs (Figure 2d). It reached 123 ± 14 beats/min (−28% of the initial value which was 447 ± 18 beats/min) after 20 min ($P < 0.01$). At the same time, the fall in heart rate was 51 ± 16 beats/min (−11% of the initial value which was 461 ± 14 beats/min) for (+)-propranolol and 68 ± 12 beats/min (−16% of the initial value which was 421 ± 19 beats/min) for pindolol ($P < 0.05$).

Systolic stroke volume Isoprenaline 1 μg induced a

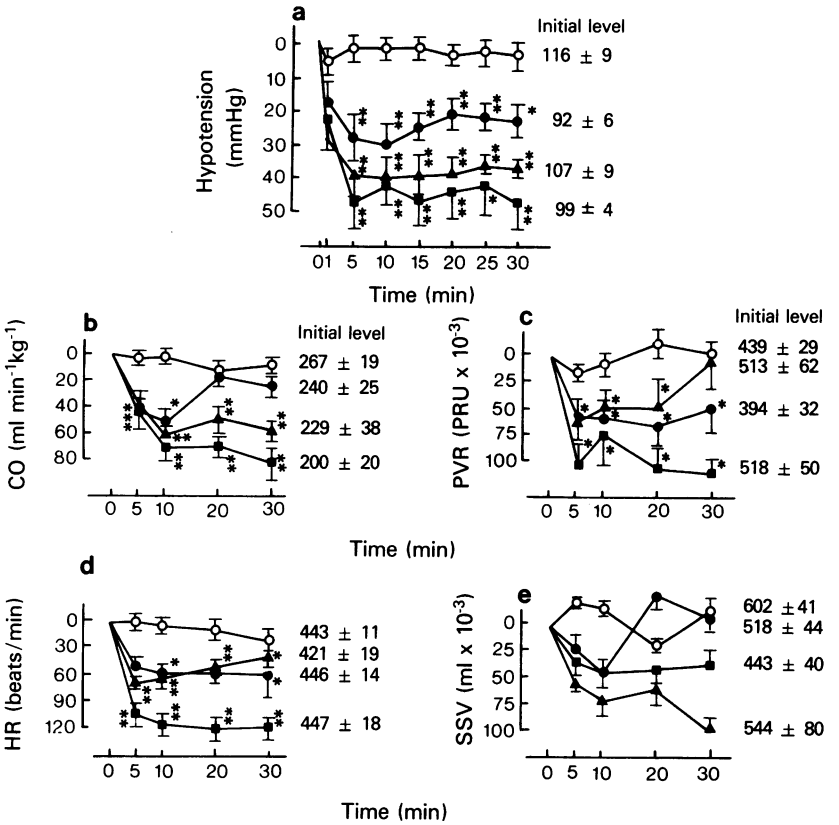

Figure 2 Changes in cardiovascular parameters after intracerebroventricular injection of 100 μg of (−)-propranolol (■); 100 μg of (+)-propranolol (●); 100 μg of pindolol (▲); control rats (○). (a) Hypotension; (b) cardiac output (CO); (c) peripheral vascular resistance (PVR); (d) heart rate (HR); (e) systolic stroke volume (SSV). Same units as in Figure 1. Differs significantly from the initial value by $*P < 0.05$; $**P < 0.01$.

progressive increase in systolic stroke volume (0.058 ± 0.02 ml (+10% of the initial value which was 0.581 ± 0.052 ml). The systolic stroke volume, remained stable during the 30 min of the experiment with 4 μg (Figure 1e).

The changes of systolic stroke volume were identical for the three β-blocking agents during the first 10 min after i.c.v. injection (Figure 2e). At the fifth min, decreases were 0.037 ± 0.010 ml (−7% of the initial value which was 0.518 ± 0.051 ml), 0.047 ± 0.012 ml (−11%, initial value: 0.443 ± 0.046 ml) and 0.054 ± 0.012 ml (−10%, initial value: 0.544 ± 0.081 ml) for (+)-propranolol, (−)-propranolol and pindolol respectively. After 20 min, this decrease remained at the same level for (−)-propranolol: 0.048 ± 0.11 ml, (−11%); with pindolol however, the decrease continued for 30 min, 0.098 ± 0.012, (18%). The systolic stroke volume returned slowly to its initial value with (+)-propranolol explaining the parallel return of the cardiac output to its normal value.

Discussion

The i.c.v. injection of (−)-propranolol and pindolol induced a similar hypotension, which was both pronounced and prolonged. With (+)-propranolol the decrease of blood pressure was significantly less. Should these differences in activity be related to the peripheral β-blocking action of the three drugs? According to Fitzgerald (1969; 1973) the relative β-blocking activity of (+)-propranolol and pindolol are 0 and 20. Thus, the (+)-isomer of propranolol, which is practically devoid of β-blocking action, induced a hypotension which was much less than its (−)-isomer. This observation is in agreement with the results obtained by Reid *et al.* (1974) with rabbits, and by Day & Roach (1974a) with rabbits and rats.

The hypotension provoked by isoprenaline, which we observed, confirmed the results obtained with dogs (Bhargava *et al.*, 1972), cats (Gagnon & Melville, 1967) and rabbits (Toda *et al.*, 1969), although a hypertensive effect was found in cats, rabbits and rats

by Day & Roach (1974a), in certain cases. These contradictory data seem to be due to variations in the experimental conditions, particularly, whether the animals used were anaesthetized or unanaesthetized (Reid *et al.*, 1974).

The decrease of blood pressure occurred during the first seconds following the injection of β-blocking drugs or of isoprenaline and persisted throughout the experiment. The rapidity of this effect may indicate a direct action on the central nervous system. Moreover we have shown (Cohen, Lindenbaum, Porquet & Wepierre, 1976) that i.c.v. injected isoprenaline partly diffused to peripheral tissues, but the blood concentration level remained too low to explain the fall in blood pressure.

Measurements of cardiac output and calculations of peripheral vascular resistance led us to define the relative roles of cardiac and vascular changes in the development of hypotension resulting from the i.c.v. administration of β-blocking agents and isoprenaline.

In the case of (−)-propranolol, (+)-propranolol and pindolol, the fall in blood pressure resulted from the simultaneous decrease of cardiac output and peripheral vascular resistance. This latter decrease was identical for the three drugs investigated. Under these conditions, the vascular effect appears not to be linked with the β-blocking action of the molecules, which was of different degree. It could therefore be attributed to other properties i.e. membrane stabilization or β-adrenergic activities.

Cardiac output, only slightly modified by (+)-propranolol, was greatly decreased by the (−)-isomer. These results led us to assume the existence of a relationship between β-blocking activity and cardiac effects. However, such a relationship was only partially verified, since pindolol and (−)-propranolol induced the same effect.

The decrease in cardiac output seemed to result from the simultaneous bradycardia, because the decrease of systolic stroke volume caused by (−)-propranolol and pindolol was very slight whilst it was absent with (+)-propranolol. This bradycardia has already been described by many authors (Kelliher & Buckley, 1970; Srivastava *et al.*, 1972; Ito & Schanberg, 1974).

Isoprenaline-induced hypotension was due solely to a fall in peripheral vascular resistance, the intensity of which was reduced by the increase in cardiac output. This latter change can be imputed to the increase in heart rate, with no change in the systolic stroke volume. These observations suggest that there are differences in reactivity between cardiac and vascular tonicity regulation centres. This double aspect of blood pressure regulation is well known, and generally it is accepted that the control centres of the medulla are differentiated and specialized to act, on the one hand, on the heart and, on the other hand, on vessels (Chai & Wang, 1962; Smith, 1965). Cardiac output and heart rate are modified in opposite ways by isoprenaline and β-blocking agents. It is therefore possible to consider a direct inhibitory or stimulatory action of these drugs on central β-receptors which might be involved in the control of cardiac activity.

The vascular effects induced by isoprenaline and β-blocking agents which are described in the present paper were the first experimental data showing the action of these drugs on vasomotor centres. However it is difficult to accept that these effects were mediated via central receptors, or at least receptors identical to those required for cardiac control, since isoprenaline and β-blocking drugs, which are competitive antagonists, induced a similar decrease in peripheral vascular resistance.

The existence of β-receptors in the control of the vasomotor centre was postulated by Day & Roach (1974a). In fact, these authors were alone in reporting opposite effects on heart rate and blood pressure of isoprenaline and β-blocking drugs. The differences observed between cardiac and vascular responses induced by central excitation showed the complexity of cardiovascular regulation in the central nervous system. These differences may be explained by postulating the existence of several distinct neurones and adrenoceptor populations (Fuxe, 1965) localized in different sites (Bradley & Dray, 1973). Finally, we might invoke, as Day & Roach (1974b) have suggested for the cat, the existence of several different types of β-receptors, which could partially explain our own results in the rat.

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