Br. J. Pharmac. (1980), **71**, 473–478

CLONIDINE DISTRIBUTION IN THE RAT: TEMPORAL RELATIONSHIP BETWEEN TISSUE LEVELS AND BLOOD PRESSURE RESPONSE

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1 The time course of the distribution of clonidine (20 μg/kg, i.v.) was determined in the rat by use of a sensitive and specific radioimmunoassay, and was compared with the hypotensive response following this dose.

2 Levels of clonidine were determined in tissues at 2 min, corresponding to the beginning of the hypotensive phase of the drug, and then at 10, 30 and 120 min during recovery of blood pressure to the pre-dose level. The peak tissue concentrations of clonidine were found at 2 min, after which they declined in a mono-exponential manner. The half-lives of clonidine in the various tissues were similar to the half-life of the recovery of blood pressure.

3 Regional variations in clonidine distribution in the brain were not very great; however, the half-life was longer in the corpus striatum and shorter in the cerebellum than in other brain regions.

4 Clonidine concentrations were highest in the kidney and similarly distributed between the cortex and medulla. Concentrations of the drug in other tissues approximated those in brain.

5 Although clonidine is thought to act primarily through the central nervous system, this distribution study shows that at the peak of the hypotensive response less than 2% of the injected dose is present in brain and at least equal concentrations of the drug are found in most peripheral tissues. Thus the possibility of peripheral mechanisms contributing to the hypotensive effect cannot be dismissed.

Introduction

Clonidine is a potent antihypertensive drug and a significant component of its hypotensive action is believed to be mediated by interaction with a population of central α-adrenoceptors, resulting in a reduction in sympathetic tone (Bolme & Fuxe, 1971; Schmitt, Schmitt & Fénard, 1971).

In addition to this central action, clonidine is known to reduce sympathetic transmission in a variety of isolated pharmacological preparations through activation of α_2-adrenoceptors on noradrenergic nerve endings (see Kobinger, 1978 for a review). However, the extent of the contribution of a peripheral action *in vivo* to the reduction in blood pressure remains unresolved.

Distribution studies give insight into possible sites of action of a drug by establishing which tissues accumulate the compound and whether the accumulation and disappearance of the compound correlates with the response produced. Because of the clinical importance of clonidine and the uncertainty as to all its sites of action, the distribution of the drug in the rat has been examined. Previous studies have used very high doses of the radioactively labelled compound (Cho & Curry, 1969; Rehbinder & Deckers, 1969). However, the availability of a highly sensitive radioimmunoassay (Jarrott & Spector, 1978) has now allowed a detailed study of both the distribution of the parent compound in peripheral and brain tissues and a comparison of the time course of the distribution with the blood pressure response after an effective hypotensive dose.

A preliminary account of this study was presented to the 12th Annual Meeting of the Australasian Society of Clinical and Experimental Pharmacologists (Conway & Jarrott, 1979).

Methods

Blood pressure experiments

Male Sprague-Dawley rats (150 to 200 g) were anaesthetized by an intraperitoneal injection of 100 mg/kg of 5-ethyl-5(1-methyl-propyl)-2-thiobarbitone sodium (Inactin), the trachea was cannulated and the common carotid artery was catheterized for monitoring

0007-1188/80/140473-06 $01.00

mean arterial pressure via a Statham pressure transducer. Blood pressure and heart rate (triggered through a Grass EKG Tachograph) were recorded on a multi-channel Grass polygraph. The jugular vein was catheterized for intravenous drug administration.

Distribution experiments

Clonidine, 20 μg/kg, was administered through the tail vein to conscious normotensive Sprague-Dawley rats (150 to 200 g). Groups of 5 animals were killed at various times after drug administration and the following organs, heart, brain, liver, kidney, lung, spleen and spinal cord were rapidly dissected, blotted, weighed and stored frozen at −20°C. Samples of small intestine, muscle and adipose tissue were also removed and frozen. When required for assay, tissues were thawed and homogenized in 4 volumes of ice-cold 0.4 M perchloric acid and centrifuged at 10,000 *g* for 4 min. The pH of the supernatants was adjusted to 7.4 with 1.0 M Tris and the volume adjusted to 10 volumes with phosphate buffered saline (PBS: 150 mM sodium chloride, 10 mM sodium phosphate, pH 7.4).

In the brain region studies, the brains were rapidly removed, placed on an ice-cold glass plate and separated into 9 regions: cerebellum, pons, medulla oblongata, hypothalamus, midbrain (including the thalamus and subthalamus), hippocampus, corpus striatum (comprising the caudate putamen and globus pallidus nuclei), n. accumbens plus olfactory tubercles and cerebral cortex (comprising the telencephalon without the striatum). The dissection procedure was based on that of Glowinski & Iversen (1966) and Horn, Cuello & Miller (1974). The samples were then treated as described above.

Clonidine estimations

Clonidine concentrations were determined by a modification of the radioimmunoassay of Jarrott & Spector (1978). In essence, another antiserum was obtained which was more specific for clonidine than the original antiserum described by these workers. This new antiserum exhibited less than 0.02% cross-reactivity for the four identified metabolites of clonidine (Jarrott, Conway & Louis, unpublished observations) and thus solvent extraction of clonidine from tissue extracts prior to radioimmunoassay was unnecessary.

Incubation tubes were set up containing tissue samples and clonidine standards (30 to 3000 pg, in samples of respective tissue from control animals). Blanks contained non-immune rabbit serum and controls contained only [^{3}H]-clonidine. After incubation with antiserum and [^{3}H]-clonidine, separation of bound and free [^{3}H]-clonidine was carried out, as described by Jarrott & Spector (1978) and the radioactivity determined by liquid scintillation spectrometry. Values of unknown samples were computed by the method of Burger, Lee & Rennie (1972).

Clonidine hydrochloride and [^{3}H]-clonidine hydrochloride (specific activity 27.6 Ci/mmol) were generously donated by Boehringer Ingelheim. The dose cited refers to the base. Tris base was obtained from Sigma Chemical Co., St. Louis and sodium chloride and sodium dihydrogen orthophosphate from Ajax Chemicals, Sydney, Australia. Perchloric acid was from Malinckrodt Chemical Works, St. Louis, Inactin from Byk Gudden, Konstanz, and normal rabbit serum from C.S.L. Labs., Melbourne, Australia.

Results

Effect of clonidine on blood pressure

Clonidine (20 μg/kg i.v.) in the anaesthetized rat produced an initial rapid but transient increase in mean arterial blood pressure followed by a more prolonged fall (Figure 1). The blood pressure rose from a control level of 143 ± 3.0 to 163 ± 3.0 mmHg, 30 s after drug administration, a rise of 14%. The nadir of 78 ± 4.9 mmHg occurred after 10 min, a fall of 45% from control levels. After 2 h the blood pressure was not significantly different from pre-injection levels.

The fall in blood pressure was accompanied by a rapid fall in heart rate, from a control level of 458 ± 10.8 to 337 ± 11.1 b.p.m., a drop of 26%. This was sustained for approximately 30 min and had recovered after 2 h.

Distribution of clonidine in major organs

Based on the blood pressure effects of clonidine, the distribution of this drug was determined 2, 10, 30 and 120 min after administration.

Clonidine was rapidly distributed in the rats, with the highest levels being present 2 min after administration in all tissues except the spleen (Table 1). The greatest concentration of clonidine was present in the kidney, this being approximately 4 times greater than the concentration in the liver at 2 min. However, there was no significant difference in the distribution of clonidine in the cortical and medullary regions of the kidney except at 2 min ($P < 0.05$, Table 1). Liver, lung and small intestine had approximately the same concentrations as did spleen, heart, and brain; adipose tissue had the lowest concentrations (Table 1).

Following the initial rapid distribution, clonidine levels then declined monoexponentially in all tissues over the time period examined. The half-lives of the

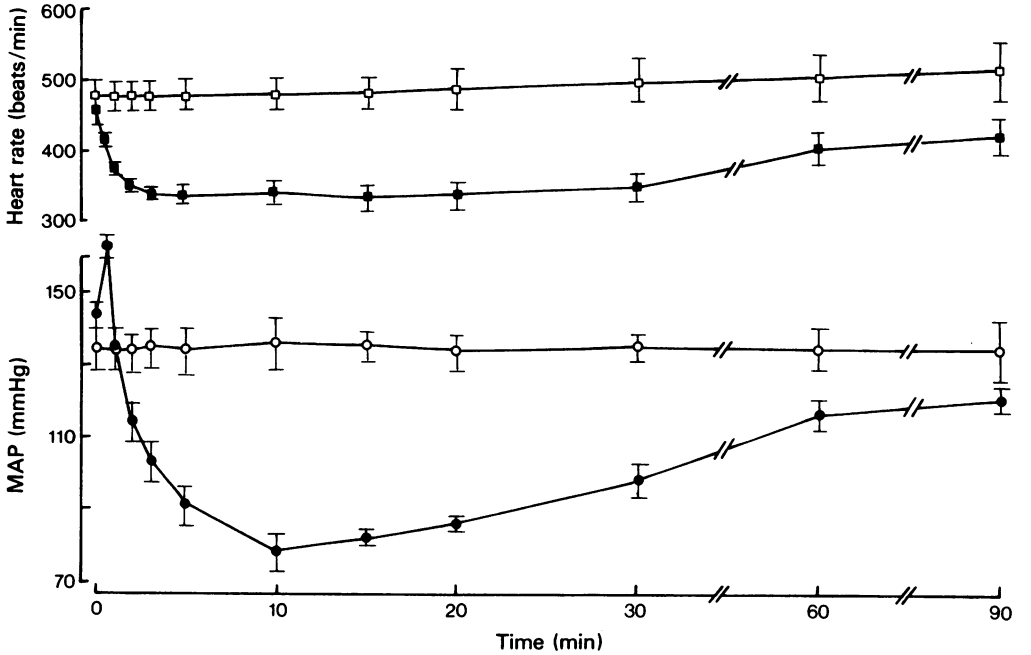

Figure 1 Changes in mean arterial pressure (MAP) and heart rate (mean values; vertical lines show s.e. mean, $n = 5$) as a function of time following intravenous administration of clonidine (20 μg/kg) in anaesthetized rats. Open symbols represent control responses and closed symbols represent drug responses.

drug in the various tissues did not differ significantly, falling within the range of 42 min (heart) to 79 min (liver) as shown in Table 2. The half-life of the decay of the fall in blood pressure (44 min) was similar to the half-life of the disappearance of clonidine from tissues.

Distribution of clonidine in brain

As in peripheral tissues, clonidine distributed rapidly throughout all regions of the brain examined. The highest concentrations occurred 2 min after drug administration in all regions except the hippocampus

Table 1 Distribution of clonidine in rat tissues at various times after intravenous administration (20 μg/kg)

Tissue	*Clonidine* (ng/g wet wt.) 2 min	10 min	30 min	120 min
Kidney	188.8 ± 15.70	123.2 ± 20.20	77.7 ± 7.54	38.0 ± 5.84
Liver	45.9 ± 3.90	36.3 ± 1.58	35.9 ± 3.87	15.7 ± 3.15
Lung	40.8 ± 4.89	27.0 ± 3.92	16.4 ± 1.17	10.8 ± 4.33
Small intestine	40.3 ± 4.71	35.5 ± 8.45	20.4 ± 2.32	8.7 ± 1.21
Spleen	33.6 ± 1.76	40.1 ± 3.65	28.2 ± 2.40	11.5 ± 2.54
Heart	32.7 ± 0.93	18.0 ± 1.05	10.8 ± 0.75	3.8 ± 0.59
Brain	25.5 ± 1.57	20.4 ± 1.32	14.7 ± 0.98	4.6 ± 0.61
Spinal cord	18.9 ± 1.82	17.3 ± 1.55	11.8 ± 1.46	5.3 ± 0.87
Muscle	18.3 ± 3.14	15.6 ± 0.62	11.4 ± 1.76	5.0 ± 0.55
Adipose	6.7 ± 0.57	5.3 ± 0.61	4.6 ± 0.42	1.0 ± 0.38
Kidney cortex	167.6 ± 13.43	109.4 ± 19.74	75.6 ± 8.14	34.0 ± 5.07
Kidney medulla	229.1 ± 20.74	148.8 ± 23.06	81.4 ± 11.28	46.6 ± 8.85

Results represent mean ± s.e. mean of 5 determinations.

where they were slightly higher at 10 min than at 2 min (Table 3).

The concentrations of clonidine in the various regions are shown in Table 3. At 10 min after the drug administration, the peak of the hypotensive response, the lowest concentration of clonidine were found in the cerebellum. These concentrations were significantly lower than in all other areas ($P < 0.01$). The highest levels at this time were present in the hippocampus, cerebral cortex and the dopamine-rich areas, the corpus striatum and the n. accumbens and olfactory tubercles (there was no significant difference between the levels in these 4 regions).

Table 2 Rate of disappearance of clonidine from rat tissues

Tissue	*Half-life of clonidine* (min)	*Upper and lower confidence limits*
Kidney	57	44–82
Liver	79	62–110
Lung	60	41–111
Small intestine	56	43–56
Spleen	66	52–88
Heart	42	35–54
Brain	50	39–68
Spinal cord	64	51–88
Muscle	67	48–107
Adipose	50	41–64

Half-lives were calculated by regression analysis; data were fitted to a natural log-linear curve. Upper and lower limits indicate 95% confidence limits for the estimated half-life.

The half-life of the disappearance of clonidine was approximately 40 to 50 min in most brain regions (Table 4). However, in the corpus striatum and the n. accumbens plus olfactory tubercles, clonidine disappeared more slowly whereas in the cerebellum the half-life was much shorter. There appeared to be some correlation between the half-lives of the drug in the various regions and the levels at 2 min although this did not reach statistical significance. ($0.1 > P > 0.05$).

Discussion

Previous studies on the distribution of clonidine in rats have involved determination of radioactivity levels following administration of the ^{14}C-labelled drug (Cho & Curry, 1969; Rehbinder & Deckers, 1969). Cho & Curry (1969) included an extraction step in their study to separate the parent compound from its metabolites, however, in the more detailed study of Rehbinder & Deckers (1969) only total radioactivity was measured. With the production of an antibody specific for the clonidine molecule (Jarrott, Conway & Louis, unpublished observations) a highly sensitive radioimmunoassay has been developed, and we have been able to study the distribution of clonidine without interference from its metabolites.

The earlier distribution studies suffered from a further disadvantage because high doses of the labelled compound were required, ranging from 100 μg/kg (Rehbinder & Deckers, 1969) to 500 μg/kg (Cho & Curry, 1969). In doses over 10 to 25 μg/kg the hypertensive effects of clonidine predominate, resulting in a delay in the blood pressure fall and a reduction in its magnitude (Hughes, 1968; Timmermans &

Table 3 Regional distribution of clonidine in brain at various times after intravenous administration (20 μg/kg)

Region	*Clonidine* (ng/g wet wt.) 2 min	10 min	30 min	120 min
Cerebellum	17.1 ± 1.90	8.6 ± 1.77	7.1 ± 0.77	0.5 ± 0.24
Medulla oblongata	20.8 ± 0.55	16.6 ± 0.81	10.2 ± 1.14	3.1 ± 0.48
Midbrain	26.1 ± 1.29	18.7 ± 1.32	11.6 ± 0.70	3.1 ± 0.61
Pons	21.1 ± 0.87	20.3 ± 1.28	12.4 ± 0.71	3.5 ± 0.70
Hypothalamus	23.5 ± 2.01	20.5 ± 0.28	12.7 ± 0.79	3.9 ± 0.68
Hippocampus	21.1 ± 1.32	24.6 ± 2.03	15.5 ± 0.56	5.7 ± 0.27
Cerebral cortex	29.3 ± 2.04	23.9 ± 1.20	18.5 ± 1.31	5.1 ± 0.71
Corpus striatum	28.9 ± 0.26	25.8 ± 1.49	19.5 ± 0.47	12.6 ± 1.13
N. accumbens & olfactory tubercles	36.6 ± 1.87	27.6 ± 1.75	20.1 ± 0.57	9.76 ± 0.71

Values represent mean ± s.e. mean of 5 determinations. One-way analysis of variance showed a significant regional variation ($F = 16.54$, $P < 0.005$). Subsequent *a posteriori* comparisons of means were performed using Duncan's multiple range test (Duncan, 1955).

Van Zwieten, 1977). The sensitivity of our radioimmunoassay allowed us to use a much lower dose of clonidine which lies within the hypotensive response range of the drug, and thus we have been able to compare the time course of the distribution with that of the antihypertensive effect.

Distribution of clonidine to all tissues of the rat investigated following an intravenous dose of 20 μg/kg was completed within 2 min as shown by the peak levels at this time. This indicated a very rapid extra-vascular distribution of this compound. The 2 min time point corresponded to the beginning of the hypotensive phase following administration of the same dose to anaesthetized rats. The blood pressure reduction reached a nadir 10 min after drug administration and recovered to control values after 90 min while tissue levels of clonidine continued to fall.

The half-life of clonidine in the various tissues was similar, varying from about 40 min in heart to 80 min in liver. These figures are slightly lower than those obtained by Cho & Curry (1969) who reported a $T_{\frac{1}{2}}$ of the order of 2 h in a number of tissues following administration of 250 μg/kg of the drug. It is interesting to note that the half-life of the recovery of blood pressure was similar to the tissue half-life of clonidine. This result suggests that the fall in blood pressure could be directly related to the concentrations of the parent drug in the organs.

Clonidine appeared to penetrate the blood brain barrier readily as the concentrations of the drug were similar to those in most peripheral tissues. This reflects the relatively high lipophilicity of clonidine (Timmermans, Brands & Van Zwieten, 1977). Regional variations in clonidine distribution in the brain were not pronounced at the peak of the hypotensive effect. Only the cerebellum had levels of the drug which were significantly lower than in other regions and the highest levels were determined in the cerebral cortex, hippocampus and the dopamine-rich areas. This variation is unlikely to be due to distribution of α_2-adrenoceptor binding sites. A recent radioligand binding study using [^{3}H]-clonidine (U'Prichard, Bechtel, Rouot & Snyder, 1979) has identified a high affinity binding site with highest levels in the cerebral cortex and lowest levels in the corpus striatum and the cerebellum, and a low affinity site with highest levels in the hypothalamus. A more probable explanation is that regional blood flow differences in brain influence the clonidine distribution.

There were some striking differences in the rate of disappearance of clonidine from the brain regions. In the corpus striatum the half-life of clonidine was more than twice that in the majority of other regions. Similarly, in the other dopamine-rich area, the nucleus accumbens plus olfactory tubercles, the rate of disappearance of clonidine was slower than the norm. From cerebellum, on the other hand, clonidine disappeared most rapidly. There also appeared to be some correlation ($0.1 > P > 0.05$) between the initial levels of the drug and the half-life in the various regions, however, this is difficult to explain. From the binding studies of U'Prichard *et al.* (1979), it seems unlikely that specific clonidine binding could explain the long half-life of clonidine in the corpus striatum although the small numbers of both high affinity and low affinity sites may contribute to the short half-life in the cerebellum. Possibly a high degree of non-specific clonidine binding occurs in the dopamine-rich areas which results in an extended half-life.

Ten minutes after the drug administration there was only 40 ng of clonidine present in the whole brain, representing less than 2% of the injected dose. Nonetheless this represents a concentration of approximately 90 pmol/g. It is known from binding studies (U'Prichard *et al.*, 1979) that the B_{max} is 1.5 to 1.8 pmol/g for the high affinity site and 11 to 12

Table 4 Rate of disappearance of clonidine from rat brain regions

Region	*Half-life of clonidine* (min)	*Upper and lower confidence limits*
Cerebellum	25	21–32
Midbrain	39	34–47
Pons	42	35–52
Medulla oblongata	43	37–53
Hypothalamus	45	39–53
Cerebral cortex	47	42–54
Hippocampus	58	51–67
N. accumbens and olfactory tubercles	68	58–81
Corpus striatum	103	85–131

Half-lives were calculated by regression analysis; data were fitted to a natural log-linear curve. Upper and lower limits indicate 95% confidence limits for the estimated half-life.

pmol/g for the low affinity site with a K_D of approximately 0.4 nM and 2.5 nM respectively. Thus if clonidine is predominantly extracellular, all binding sites are probably saturated at this time.

This study shows that the concentration of clonidine in most peripheral tissues is at least equal to the concentration in brain following a single intravenous dose. The possibility therefore exists that peripheral mechanisms such as a reduction of renin secretion, either directly or indirectly, and a reduction in vascular tone and reactivity (see Schmitt (1977) for a review) may contribute to the hypotensive effect of the drug.

We would like to thank Dr G. Hutchinson for expert assistance in conducting the blood pressure experiments.

This work was supported by a grant from the National Health and Medical Research Council of Australia.

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(Received December 12, 1979. Revised June 19, 1980.)